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WITNESS my hand this
Eighth day of September 2003

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PROVISIONAL SPECIFICATION

Applicants:

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

Invention Title:

METHODS FOR THE CHEMICAL AND PHYSICAL MODIFICATION OF NANOTUBES, METHODS FOR LINKING THE NANOTUBES, METHODS FOR THE DIRECTED POSITIONING OF NANOTUBES, AND USES THEREOF

The invention is described in the following statement:

METHODS FOR THE CHEMICAL AND PHYSICAL MODIFICATION OF NANOTUBES, METHODS FOR LINKING THE NANOTUBES, METHODS FOR THE DIRECTED POSITIONING OF NANOTUBES, AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to methods for chemically and physically modifying nanotubes with nucleic acid, and uses thereof. The invention also relates to linked nanotubes, in particular methods for controlling the linking of such nanotubes involving the use of DNA, and uses thereof. The invention also relates to devices and applications which require the placement of nanotubes in specific locations, in particular methods for controlling the directed positioning of such nanotubes involving DNA, and uses thereof.

BACKGROUND OF THE INVENTION

Nanotubes are typically small cylinders made of organic or inorganic materials. For example, known types of nanotubes include peptidyl nanotubes and carbon nanotubes.

Carbon nanotubes are cylindrical shells of graphitic sheets typically having diameters of 1-300 nanometers and lengths of 1-100μm and sometimes up to mm in size. They offer unique physical properties that are potentially useful in a variety of nanometer-scale devices and technologies. Indeed, carbon nanotubes have been proposed as new materials for electron field emitters in panel displays, single-molecular transistors, scanning probe microscope tips, gas and electrochemical energy storage, catalyst and protein/DNA supports, molecular-filtration membranes, and energy-absorbing materials (see, for example, Dekker, "Carbon nanotubes as molecular quantum wires," Physics Today, May 1999, M. Dresselhaus, *et al.*, *Phys. World*, January, 33, 1998; P.M. Ajayan, and T.W. Ebbesen, *Rep. Prog. Phys.*, 60, *1027*, 1997; R. Dagani, *CE News*, January 11, *31*, 1999).

However, most of the envisioned applications

require that the nanotubes are grown in a highly controlled fashion, i.e., with their diameter, length, location and microstructure, controllable and reproducible. Unfortunately, carbon nanotubes synthesised by most of the common techniques, such as arc discharge and catalytic pyrolysis, often exist in a randomly entangled state (see, for example, T.W. Ebbesen and P.M. Ajayan, *Nature* 358, *220*, 1992; M. Endo *et al.*, *J. Phys. Chem. Solids*, 54, 1841, 1994; V. Ivanov *et al.*, *Chem. Phys. Lett.*, *223*, 329, 1994). In recent times, these problems have largely been overcome by preparing aligned carbon nanotubes either by post-synthesis manipulation (see, for example, M. Endo, *et al.*, *J. Phys. Chem.* solids, 54, *1841*, 1994; V. Ivanov, *et al.*, *Chem. Phys. Lett.*, 223, *329*,1994; H Takikawa, *et al.*, *Jpn. J. Appl. Phys.*, 37, *L187*, 1998), or by synthesis-induced alignment (see, for example, W. Z. Li, *Science*, 274, *1701*,, 1996; Che. G., *Nature*, 393, *346*, 1998; Z. G. Ren, *et al.*, *Science*, 282, *1105*, 1998; C. N., Rao, *et al.*, *J. C.S., Chem. Commun.*, 1525 , 1998).

Aligned nanotubes have also been synthesised using porous templates (W. Z. Li *et al.*, "Large Scale Synthesis of Aligned Carbon Nanotubes," Science, Vol. 274, 1701 (1996); S. Fan *et al.*, "Self-oriented regular arrays of carbon nanotubes and their field emission properties," Science, Vol. 283, 512 (1999); J. Li *et al.*, "Highly ordered carbon nanotubes arrays for electronic applications", Appl. Phys. Lett., Vol. 75, 367 (1999)). Other papers on growing aligned nanotubes have described DC plasma assisted hot filament deposition (Z. F. Ren *et al.*, "Synthesis of large arrays of well-aligned carbon nanotubes on glass," Science, Vol. 282, page 1105 (1998)).

Despite all of the developments in the growth of nanotubes, some of the more useful applications for this technology remain elusive, as they require not only regular growth of nanotubes, but also the linking of the nanotubes either side-to-side or end-to-end.

The Applicant has now developed a process capable of linking nanotubes. Importantly, the Applicant has developed a process of chemically attaching nucleic acid molecules to such nanotubes, either on the walls or on the tips, in a controlled manner, which allows linkage of nanotubes either side-to-side or end-to-end, thereby dramatically increasing their usefulness. The Applicant has also developed a process of physically attaching nucleic acid molecules to the walls of such nanotubes, which preserves the sp^2 structure of the nanotubes and thus their electronic characteristics. The Applicant has also developed a method for locating nanotubes to specific targets using DNA-directed self-assembly.

SUMMARY OF THE INVENTION

In its broadest aspect, the invention provides a method of chemically attaching nucleic acid molecules to one or more nanotubes. The invention also provides a method of physically attaching nucleic acid molecules to one or more nanotubes. The invention also provides a method of linking these nanotubes. Further, the invention provides a process whereby nanotubes may be directed to specific locations.

Accordingly, in a first aspect, the present invention provides a nanotube with one or more nucleic acid molecule(s) attached thereto.

In a second aspect, the invention provides a method of chemically modifying a nanotube comprising the steps of:

a) chemically attaching at least one linker to said nanotube, wherein said linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said nanotubes via said functional group on said linker; or

c) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*,

starting from said functional group on said linker.

In a third aspect, the invention provides a method of chemically modifying a nanotube comprising the steps of:

a) photochemically attaching at least one linker to said nanotube, wherein the linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said nanotubes via said functional group on said linker; or

c) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said linker.

In a fourth aspect, the invention provides a method of physically modifying a nanotube comprising the steps of:

a) physically adsorbing at least one anchor, wherein said anchor consists wholly or partly of a functional group to the surface of said nanotube; and

b) chemically attaching one or more nucleic acid molecules to said functional group on said anchor adsorbed on the nanotube; or

c) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said anchor.

In a fifth aspect, the invention provides a plurality of linked nanotubes.

In a sixth aspect, the present invention provides a method of linking nanotubes comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to a first nanotube; and

b) hybridising the first nucleic acid molecule with a second nucleic acid molecule of a second base sequence attached on a second nanotube, wherein the base sequence of the second nucleic acid molecule is substantially complementary to the base sequence of the

first nucleic acid molecule.

In a seventh aspect, the present invention provides a method of linking nanotubes comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to said nanotubes; and

b) hybridising the first nucleic acid molecule with a second nucleic acid molecule which comprises a base sequence substantially complementary to the first base sequence and further comprises a second or a third base sequence which is/are not complementary to the first base sequence, but is/are complementary to each other.

In an eighth aspect, the present invention provides a method of linking nanotubes comprising the steps of:

a). attaching a first nucleic acid molecule of a first base sequence to a first nanotube;

b). attaching a second nucleic acid molecule of a second base sequence to a second nanotube;

c). hybridising the first nucleic acid molecule to a third nucleic acid molecule which comprises a base sequence substantially complementary to the base sequence of the first nucleic acid molecule and which further comprises at least 5 nucleotides which are not complementary to the base sequence of the first or second nucleic acid molecules;

d). hybridising the second nucleic acid molecule to a fourth nucleic acid molecule which comprises a base sequence substantially complementary to the base sequence of the second nucleic acid molecule and which further comprises at least 5 nucleotides which are not complementary to the base sequences of the first or second nucleic acid molecules;

wherein the base sequences of the third and fourth nucleic acid molecules are substantially complementary such that under stringent hybridisation conditions said third and

fourth nucleic acid molecules hybridise thereby linking said first and second nanotubes.

In a ninth aspect, the present invention further provides linked nanotubes produced by the method of the sixth, seventh and eighth aspects linked together to form a string of linked nanotubes. The method of linking being the same method as that disclosed in the sixth, seventh and eighth aspects, respectively, but subsequently repeated.

In a tenth aspect, the present invention provides a method of linking nanotubes comprising the steps of:

a). providing a plurality of nanotubes with attached nucleic acid molecules, wherein said nucleic acid molecules have the same or different base sequences;

b). exposing said nanotubes to a nucleotide strand which comprises a base sequence substantially complementary to one or more of the base sequences of said nucleic acid molecules; and

c). incubating said nanotubes and nucleotide strand under appropriate hybridisation conditions wherein said nanotubes are linked via hybridisation of the nucleic acid molecules with the nucleotide strand.

In an eleventh aspect, the present invention provides a method for directing nanotubes to specific targets comprising the steps of:

a). attaching a first nucleic acid molecule of a first base sequence to said nanotubes;

b). attaching a second nucleic acid molecule of a second base sequence which is substantially complementary to the first base sequence to a target; and

c). hybridising said first and second nucleic acid molecules.

In a twelfth aspect, the present invention provides a method for directing nanotubes to specific targets comprising the steps of:

a). attaching a first nucleic acid molecule of

a first base sequence to a nanotube;

b). attaching a second nucleic acid molecule of a second base sequence to a target;

c). exposing said nanotube and target to a third nucleic acid molecule which comprises a base sequence which is substantially complementary to both the first and second nucleic acid molecules; and

d). incubating said nanotube and target under appropriate hybridisation conditions wherein said nanotube and target are linked via hybridisation of the first and second nucleic acid molecule via the third nucleic acid molecule.

In a thirteenth aspect, the present invention provides a method for directing nanotubes to specific targets comprising the steps of:

a). attaching a first nucleic acid molecule of a first base sequence to a nanotube;

b). attaching a second nucleic acid molecule of a second base sequence to a target;

c). hybridising the first nucleic acid molecule to a third nucleic acid molecule which comprises a base sequence substantially complementary to the base sequence of the first nucleic acid molecule and which further comprises at least 5 nucleotides which are not complementary to the base sequence of the first or second nucleic acid molecules;

d). hybridising the second nucleic acid molecule to a fourth nucleic acid molecule which comprises a base sequence substantially complementary to the base sequence of the second nucleic acid molecule and which further comprises at least 5 nucleotides which are not complementary to the base sequences of the first or second nucleic acid molecules;

wherein the base sequences of the third and fourth nucleic acid molecules are substantially complementary such that under stringent hybridisation conditions said third and fourth nucleic acid molecules hybridise thereby directing

said nanotube to said target.

In a fourteenth aspect, the present invention provides a method for directing nanotubes to specific targets comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to said nanotubes;

b) attaching a second nucleic acid molecule of a second base sequence to a target, where the second base sequence is not complementary to the first base sequence, and where the second base sequence may or may not be the same as the first base sequence, and

c) adding a third nucleic acid molecule which has in one part a base sequence substantially complementary to the base sequence of the first nucleic acid molecule and in another part a base sequence substantially complementary to the base sequence of the second nucleic acid molecule; and

d) hybridising the third nucleic acid molecule to the first and the second nucleic acid molecules, thus linking the nanotube to the target.

In a fifteenth aspect, the invention provides a nucleic acid sensor comprising a nanotube with one or more nucleic acid molecule(s) attached thereto, wherein the base sequence of the said attached nucleic acid molecule is substantially complementary to all or a portion of the base sequence of the nucleic acid molecules being detected.

In a preferred embodiment, the sensor consists of an array of groups of one or more nanotubes, each group having one or more nucleic acid molecules of the same base sequence attached to each nanotube in the group, and where the base sequence of the nucleic acid molecules attached to the nanotubes in one group differs from those in other groups so that a number of different target DNA molecules may be detected.

In a sixteenth aspect, the invention provides an actuator comprising one or more nanotubes with one or more

nucleic acid molecule(s) attached thereto and a membrane support to which the DNA-modified nanotubes are attached.

In a seventeenth aspect there is provided a conductor comprising one or more nanotubes with one or more nucleic acid molecule(s) attached thereto.

In one preferred embodiment, the conductor is a nanowire comprised of nanotubes linked together via nucleic acid hybridisation. Preferably, the nanowire further comprises nanoparticles or coating of conductive material.

The nanotubes may be made from any suitable material already known in the art. Preferably, the nanotubes are carbon nanotubes. The carbon nanotubes may be grown using any known procedure in the art; for example, Arc discharge method, chemical vaporisation deposition method (CVD), plasma enhanced chemical vaporisation deposition method (PECVD), laser ablation/vaporization, pyrolysis, thermal chemical vapour deposition, electrolysis, flame synthesis, or a combination of these techniques for the manufacture of either multi-walled nanotubes (MWNTs) or single-walled nanotubes (SWNTs). The type of nanotube used depends partly on the end use of the nanotube. For example, the nanotubes may be aligned, aligned and patterned, or dispersed nanotubes. The nanotubes may also be SWNTs or MWNTs.

Each of the first, second, third or fourth nucleic acid molecules may be DNA, cDNA, RNA, oligonucleotide, oligoribonucleotide, modified oligonucleotide, modified oligoribonucleotide, peptide nucleic acid (PNA), or hybrid molecules thereof. Preferably, the nucleic acid molecule is an oligonucleotide, oligoribonucleotide or RNA-DNA hybrid molecule thereof. Most preferably, the nucleic acid molecule is an oligonucleotide.

The nucleic acid molecule may be synthesised in a DNA synthesiser or produced by enzymatic digestion or

enzymatic polymerisation and then attached on to the nanotube by any method known including by reacting the nucleic acid molecule with a nanotube modified with a functional group or with a nanotube physically modified with an anchor containing a functional group. Alternatively, the nucleic acid molecule may be synthesised *in situ* onto a functionalised nanotube or onto a nanotube physically modified with an anchor containing a functional group.

In one embodiment, the synthesised nucleic acid molecule is attached to a nanotube modified with carboxyl groups either by oxidation or by photo-irradiation of an azido linker containing carboxyl groups. The carboxyl group on the nanotube or on the azido linker forms an amide bond with 5' or 3'amino modified DNA. This amide bond might be extended by incorporating a spacer between the DNA and the linker by using difunctional reagents such as standard amino acids or non-standard amino acids (C3-C12), for example, 11-amino undecanoic acid and peptides.

In another embodiment the nucleic acid molecule is synthesised *in situ* by either oxidizing nanotubes to form hydroxyl groups or attaching functional hydroxyl groups to the nanotubes using photochemical reaction of azido compounds such as azido thymidine or azidoadenosine via the azide functional group to the nanotube. The DNA molecule is built up by sequentially adding nucleotides by phosphoramidite chemistry used in automated DNA synthesis.

In yet a further embodiment, the DNA is physically attached to the nanotube via a covalent linkage to an anchor which is physically adsorbed to the surface of the nanotube. The DNA may be pre-synthesised or synthesised *in situ*. The anchor typically contains a hydrophobic domain such as a pyrenyl, porphyrin or acridine derivative which interact strongly with the hydrophobic walls of the nanotube, and a functional group to which the DNA can be attached or built-up from. Alternatively the anchor may be an oligonucleotide spacer

such as oligo thymidine or oligo guanidine which physically adsorbs to the nanotube walls and from which extends the hybridizing DNA.

The nucleic acid molecule may be attached to the walls(s) or side(s) and/or the tip(s) of the nanotube.

The carbon nanotubes can be linked end-to-end, side-to-side, or combinations thereof and the linking process utilises the unique self-annealing properties of nucleic acids. In one embodiment, the linking process involves the attachment of a single-stranded nucleic acid molecule to the side or end of a first nanotube, and the attachment of a complementary single-stranded nucleic acid molecule to the side or end of a second nanotube, wherein, under appropriate hybridisation conditions, the nucleic acid molecules hybridise together thereby linking the nanotubes.

In another embodiment, the linking process involves the attachment of a first single-stranded nucleic acid molecule to the side or end of carbon nanotubes. As all of the carbon nanotubes comprise the same single-stranded nucleic acid, there is no self-annealing. The nanotubes are then exposed to a second nucleic acid molecule, which comprises a segment of single-stranded nucleic acid, which is complementary to the first nucleic acid molecule. The second nucleic acid molecule also comprises a further segment of nucleic acid sequence which is either the "positive" strand or "negative" (complementary) strand of nucleic acid. Accordingly, in this embodiment, the complementary strands of the first and second nucleic acids hybridise and then the positive and negative strands of the second nucleic acid, which are complementary, hybridize thereby linking the nanotubes by a "bridge structure". This "bridge structure" may be used to increase the distance between nanotubes without requiring the synthesis of long strands of nucleic acid, which may suffer problems of self-complementarity and the like. It will be appreciated by those skilled in the art

that further variations on this embodiment may be created such as the second nucleic acid having non-complementary nucleic acid segments, but having complementary sequences with a third, fourth or more nucleic acid molecules.

In a further embodiment, the nanotubes may be linked by hybridising nanotubes comprising a first attached nucleic acid molecule to a second nucleic acid molecule, which comprises two or more complementary nucleic acid sequences as contiguous repeats or non-contiguous repeats. Under appropriate hybridisation conditions a string of nanotubes, joined via the second nucleic acid molecule, is produced.

The linked carbon nanotube may also include other nanoparticles including spheres, rods, octahedrons, which may be made of any material including transition metals, for example, gold, silver, and cadmium sulphide (CdS). The other nanoparticles may be incorporated in and/or coated on to the nanotube, nucleic acid molecule, nanotube with the attached nucleic acid molecule and/or the linked nanotube.

BRIEF DESCRIPTION OF THE SCHEMES

Scheme 1 shows chemical attachment of DNA to nanotubes via hydroxyl groups and (a) DNA synthesis *in situ* or (b) attachment of pre-synthesised DNA.

Scheme 2 shows chemical attachment of DNA to nanotubes via carboxyl groups and (a) attachment of pre-synthesised DNA or (b) DNA synthesis *in situ*.

Scheme 3 shows photochemical modification of nanotubes via azidothymidine and (a) DNA synthesis *in situ* or (b) attachment of pre-synthesised DNA.

Scheme 4 shows photochemical modification of nanotubes via photo-etching linkers and (a) attachment of pre-synthesised DNA or (b) DNA synthesis *in situ*.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic diagram of gold

nanoparticles functionalised with DNA. The gold nanoparticles are depicted by balls, and the oligonucleotides by black lines.

Figure 2 shows a TEM image of gold nanoparticles functionalised with DNA. The DNA cannot be seen in this image.

Figure 3 shows TEM images of multi-walled nanotubes and gold nanoparticles with and without DNA, showing hybridization of DNA-modified gold nanoparticles to DNA chemically attached to the walls of MWNTs, and controls. The DNA has been chemically attached to the MWNTs by a photochemical reaction of azidothymidine with the nanotubes, followed by *in situ* synthesis of DNA using phosphoramidite chemistry. The DNA (NT3') chemically attached to the nanotubes, and its complementary DNA' (Gold2A-SH3') bound to the gold nanoparticles, each are 16 nucleotides long. (a) NT-DNA + Gold-DNA', (b) NT-DNA + Gold, (c) NT + Gold-DNA' (d) NT + Gold.

Figure 4 shows TEM images of multi-walled nanotubes and gold nanoparticles with and without DNA, showing hybridization of DNA-modified gold nanoparticles to DNA chemically attached to the walls of MWNTs, and controls. The DNA is chemically attached to the MWNTs by a photochemical reaction of azidothymidine with the nanotubes, followed by *in situ* synthesis of DNA using phosphoramidite chemistry. Here, the DNA (NT3'-T_{19}) chemically attached to the carbon nanotubes is 35 nucleotides long. The DNA bound to the gold nanoparticles is either 16 (Gold2A-SH3') or 35 (Gold3A-SH3') nucleotides long. The 16-nucleotide NT3' portion of NT3'-T_{19} has a base sequence which is complementary to Gold2A-SH3', and to 16 nucleotides of the 35-nucleotide Gold3A-SH3'. (a) NT-DNA + Au-Gold2A-SH3' (sample I), (b) NT + Au-Gold2A-SH3' (sample L), (c) NT-DNA + Au-Gold3A-SH3' (sample J), (d) NT + Au-Gold3A-SH3' (sample M), (e) NT-DNA + Au (sample K), (f) NT + Au (sample N).

Figure 5 shows TEM images of single-walled

nanotubes and gold nanoparticles with and without DNA, showing hybridization of DNA-modified gold nanoparticles to DNA chemically attached to SWNTs, most probably at their tips, plus controls. Here, pre-synthesised DNA with an amine linker on its 3′ end is chemically attached through an amide bond to the SWNTs which had been functionalised with carboxyl groups. The DNA (NT3′-$NH_2$3′) attached to the nanotubes is 16 nucleotides long. The DNA′ (Gold2A-SH3′) bound to the gold nanoparticles is also 16 nucleotides long and has a base sequence complementary to that of NT3′. (a) NT-DNA + Au-DNA′ (sample C), (b) NT-DNA + Au (sample D), (c) NT + Au-DNA′ (sample E), (d) NT + Au (sample F).

Figure 6 shows TEM images showing hybridization of DNA-modified gold nanoparticles to DNA physically attached to MWNTs (sample 1) and to DNA chemically attached to MWNTs (sample 2), and controls. Here, the DNA (NT3′-T19) synthesized *in situ* in the presence of sample 1 (unmodified, aligned MWNT) and sample 2 (aligned MWNT modified by a photochemical reaction with azidothymidine) is 35 nucleotides long. Sample 3 (unmodified, aligned MWNT) has not been subjected to DNA synthesis. The 16-nucleotide DNA molecule (Gold2A-SH3′) attached to the gold nanoparticles has a base sequence complementary to the 16-nucleotide NT3′ portion of NT3′-T19. (a) sample 1 with gold nanoparticles modified with Gold2A-SH3′ (b) sample 1 with gold nanoparticles alone, (c) sample 2 with gold nanoparticles modified with Gold2A-SH3′, (d) sample 2 with gold nanoparticles alone, (e) sample 3 with gold nanoparticles modified with Gold2A-SH3′, (f) sample 3 with gold nanoparticles alone.

ABBREVIATIONS USED

AZT	Azidothymidine
CPG	Controlled Pore Glass
DMF	N,N,Dimethylformamide
DCM	Dichloromethane

DIEA	N,N,Diisopropylethylamine
EDTA	Ethylenediaminetetraacetic acid
Fmoc-Cl	Fluorenylmethoxycarbonyl chloride
Fmoc	Fluorenylmethoxycarbonyl
Fmoc-HDA	N-Fluorenylmethoxycarbonyl-1,6-diaminohexane
HSN	N-hydroxysuccinimide
PECVD	Plasma Enhanced Chemical Vapour Deposition
MWNT	Multi-walled nanotube
SWNT	Single-walled nanotube
PNA	Peptide nucleic acid
TEM	Transmission Electron Microscopy
XPS	X-ray Photoelectron Spectroscopy

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention employs, unless otherwise indicated, conventional molecular biology or chemistry techniques within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature. *See*, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "Immobilised Cells and Enzymes" (IRL Press, 1986); B. Perbal, "A Practical Guide to Molecular Cloning" (1984); Sambrook, *et al.*, "Molecular Cloning: a Laboratory Manual" (1989); Ausubel, F. *et al.*, 1989-1999, "Current Protocols in Molecular Biology" (Green Publishing, New York); P.Y. Brucce, "Organic Chemistry" (1995); and J. McMurry, "Organic Chemistry" (1988).

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents

forms part of the common general knowledge in the art, in Australia or in any other country.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

According to one aspect of the invention, carbon-containing material is formed into carbon nanotubes and then one or more nucleic acid molecules are attached thereto.

Any means of growing carbon nanotubes may be used.

The carbon-containing material may be any compound or substance which includes carbon and which is capable of forming carbon nanotubes when subjected to pyrolysis in the presence of a suitable catalyst. Examples of suitable carbon-containing materials include alkanes, alkenes, alkynes or aromatic hydrocarbons and their derivatives, for example, methane, acetylene, benzene, transition metal phthalocyanines, such as Fe (II) phthalocyanine, and other organometallic compounds such as ferrocene and nickel dicyclopentadiene.

The catalyst may be any compound, element or substance suitable for catalysing the conversion of a carbon-containing material to aligned or dispersed carbon nanotubes under pyrolytic conditions. The catalyst may be a transition metal, such as Fe, Co, Al, Ni, Mn, Pd, Cr or alloys thereof in any suitable oxidation state.

The catalyst may be incorporated into the substrate or may be included in the carbon-containing material. Examples of carbon-containing materials which include a transition metal catalyst are Fe (II) phthalocyanine, Ni (II) phthalocyanine, nickel dicyclopentadiene and ferrocene. When the catalyst and carbon-containing material are included in the same material it may be necessary to provide sources of additional catalyst or additional carbon-containing

material. For example, when ferrocene is used as the catalyst and a source of carbon, it is necessary to provide an additional carbon source, such as ethylene, to obtain the required nanotube growth.

The pyrolysis condition employed will depend on the type of carbon-containing material employed and the type of catalyst, as well as the length and density of the nanotubes required.

In this regard it is possible to vary the pyrolysis conditions, such as the temperature, time, pressure or flow rate through the pyrolysis reactor, to obtain nanotubes having different characteristics.

For example, performing the pyrolysis at a higher temperature may produce nanotubes having different base-end structures relative to those prepared at a lower temperature. The pyrolysis will generally be performed within a temperature range of 800°C to 1100°C. Similarly lowering the flow rate through a flow-type pyrolysis reactor may result in a smaller packing density of the nanotubes and vice versa. A person skilled in the art would be able to select and control the conditions of pyrolysis to obtain nanotubes having the desired characteristics.

The diameter of nanotubes is controllable by selecting a particular catalyst layer thickness. For example, by varying the thickness of a cobalt layer from 2 nm to 60 nm, the nanotube diameter goes from about 30 nm to about 150 nm. The size of the catalyst islands is determined, as least in part, by the thickness of the catalyst layer, with thin layers leading to smaller diameter islands, and thicker layers leading to larger diameter islands. The range of nanotube diameters typically attainable is 10 to 300 nm. Control runs are easily performed to determine an appropriate catalyst layer thickness for a desired nanotube diameter.

The nanotube length is primarily controlled by the duration of the high frequency PECVD process, but not

in a monotonically linear fashion. There are three stages of the process as it affects length - growth, stability, and etch. Specifically, length initially increases for a certain time period (about 5 minutes from the initiation of the process). This growth stage is followed by a period of substantially slowed growth - the stability stage. And then the nanotubes begin to be etched away such that the length is reduced - the etch stage. It appears that at some point during nanotube growth catalyst particles become completely encased by graphitic shells. Once the catalyst is so encased, nanotube growth slows (stability stage), and the etching character of the high frequency PECVD process begins to predominate (etch stage). It is also possible that the increasing length of the nanotubes interferes with the ability of reactive species to reach the catalyst at the bottom of the growing tube, thereby slowing the growth. Thus, for a given set of high frequency PECVD process parameters, the duration will typically be chosen to attain a desired length, without entering into the etch stage. However, it is possible to reach any of the three stages, and it is possible for certain advantages to exist in each. For example, it is possible that moving at least partially into the etch stage will provide nanotubes with open, as opposed to capped, ends, which may be desirable for some applications. Control runs are easily performed to find a suitable process duration to provide a desired length.

In one particularly preferred embodiment aligned, multi-walled carbon nanotubes are grown by pyrolysis of iron(II) phthalocyanine (FePc) under Ar/H_2 at 800-1100 °C (Li, D.-C., Dai, L., Huang, S., Mau, A.W.H. and Wang Z.L. (2000) Chem. Phys. Lett, 316, 349-355).

Commercial suppliers of SWNTs made by continuous process include Carbon Nanotechnologies Inc. (Houston, Texas). Commercial suppliers of SWNTs made by CVD include Iljin Nanotech Co. Ltd. (Korea). Commercial suppliers of MWNTs made by CVD include Iljin Nanotech Co. Ltd. (Korea).

Having obtained the carbon nanotubes, one or more nucleic acid molecules are attached, preferably via a linker resulting in a covalent coupling, or by an anchor resulting in physical attachment.

The term "linker" includes compounds or molecules which are composed wholly or partly of at least one functional group that are capable of linking the nanotube directly to the nucleic acid molecule.

Suitable linkers for chemical or photochemical attachment include photoetchable linkers, for example, azido compounds such as azido-thymidine, azido adenosine, azido-nitrobenzyloxy succinimide, azidophenyl isothiocyanate, 4-(P-Azido salicylamido) butylamine, 4-(P-Azido salicylamido) butyl-3' (2'-pyridylthio)propionamide, 4-(P-Azido salicylamido) butyl-maleimide propionamide, and p-azidophenyl glyoxal monohydrate.

It will be appreciated that the linker and the nucleic acid molecule may be optionally interrupted by a spacer.

The term "spacer" includes compounds or molecules that extend the nucleic acid molecule away from the surface of the nanotube and allow for easier hybridization to complementary nucleic acid molecules.

Suitable spacer groups for extending the hydroxyl group either directly attached to the nanotube or on azido compounds photoetched to the nanotubes are described by formula I:

W-S-X

I

wherein W is COOH, $(-CO)_2O$, COCl, halide, SO_3H or $ClSO_2$;

S is H, alkyl$(CH_2)_{1-12}$, aryl, aralkyl, cycloalkyl or polyalkyether; and

X is COOH, COCl, halide, SO_3H, $ClSO_2$, CHO, NCO, NCS, SH, OH or maleimide.

Suitable spacer groups for extending the carboxyl group either directly attached to the nanotube or on azido compounds photoetched to the nanotubes are described by formula Ia:

$$W_a\text{-}S_a\text{-}X_a$$

Ia

wherein W_a is NH_2, OH, SH or halide;

S_a is H, alkyl$(CH_2)_{1\text{-}12}$, aryl, aralkyl, cycloalkyl or polyalkylether; and

X_a is COOH, COCl, halide, SO_3H, $ClSO_2$, CHO, NCO, NCS, SH, OH or maleimide.

The term "anchor" includes compounds or molecules with a high affinity for physical adsorption to the surface of the nanotubes.

Suitable anchors for physical attachment to the nanotubes include pyrenebutanoic acid succinimide ester to which the DNA can be attached, acridine phosphoramidite from which the DNA can be built, or a fluorescein derivative from which the DNA can be built or to which the DNA can be covalently attached. Alternatively, the anchor may be an oligonucleotide spacer such as oligo thymidine or oligo guanidine which physically adsorbs to the nanotube walls and from which extends the hybridizing DNA.

The term "nucleic acid" is synonymous with DNA, RNA, and polynucleotides. A "nucleic acid molecule" or "polynucleic acid molecule" refers herein to deoxyribonucleic acid and ribonucleic acid in all their forms, i.e., single and double-stranded DNA, cDNA, mRNA, and the like, which may include modified bases. In one preferred embodiment, the nucleic acid is an oligonucleotide, oligoribonucleotide or an RNA-DNA hybrid molecule comprising nucleotides which may be substituted or modified in their sugar, base or phosphate group.

The nucleotides may be in the form of deoxyribonucleotides, ribonucleotides,

deoxyribonucleotide-ribonucleotide hybrids, or derivatives thereof as herein described. Modified bases, sugars or phosphate linkages of nucleotides, such as phosphoramidate, or phosphorothioate linkages in the sugar phosphate chain, may also provide resistance to nuclease attack. Binding affinity may also be optimized in particular circumstances, by providing nucleotides solely in the form of nucleotides, ribonucleotides, deoxyribonucleotides, or combinations thereof.

If the nucleic acid is an oligonucleotide then the respective 3' and 5' termini of the oligonucleotides or alternatively the 3' and 5' end termini, may be modified to stabilise the nucleic acid from degradation. For example, blocking groups may be added to prevent terminal nuclease attack, in particular 3'-5' progressive exonuclease activity. By way of example, blocking groups may be selected from substituted or unsubstituted alkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkanoyl. Substituents may be selected from C_1-C_5 alkyl; halogens such as F, Cl or Br; hydroxyl; amino; C_1-C_5 alkoxy and the like. Alternatively, nucleotide analogues such as phosphorothioates, methylphosphonates or phosphoramidates or nucleoside derivatives (such as alpha-anomer of the ribose moiety) which are resistant to nuclease attack may be employed as terminal blocking groups. The blocking group may be an inverted linkage such as a 3' 3' thymidine linkage or a 5' 5' pyrophosphate linkage as in the guanosine cap.

Alternatively, groups that alter the susceptibility of the nucleic acid molecule to other nucleases may be inserted into the 3' and/or 5' end of the nucleic acid molecule. For example, 9-amino-acridine attached to the nucleic acid molecule may act as a terminal blocking group to generate resistance to nuclease attack on the nucleic acid molecule.

It will be readily appreciated that a variety of other chemical groups, e.g. spermine or spermidine could

be used in a related manner.

The nucleic acid molecule of this invention may be produced by nucleic acid synthetic techniques that are known in the art, and then attached to the nanotube, or synthesised *in situ*. For example, DNA can be prepared by a method such as the phosphotriester method of Narang *et al.*, 1979, Meth. Enzymol. 68:90-99; the phosphodiester method of Brown *et al.*, 1979, Meth. Enzymol. 68:109-151; the diethylphosphoramidite method of Beaucage *et al.*, 1981, Tetrahedron Lett. 22:1859-1862; the triester method of Matteucci *et al.*, 1981, J. Am. Chem. Soc. 103:3185-3191 or automated synthesis methods; and the solid support method of U.S. Pat. No. 4,458,066, which publications are each incorporated herein by reference. Synthetic procedures generally involve the sequential coupling of activated and protected nucleotide bases to give a protected nucleotide chain, whereafter protecting groups may be removed by suitable treatment. Preferably the compounds will be synthesized on an automated synthesiser such as those made by Applied Biosystems (a Division of Perkin Elmer), Pharmacia or Millipore.

In addition to being synthesized chemically, nucleic acid molecules with modified nucleotides may be synthesized enzymatically. The phosphodiester bonds of RNA can be replaced by phosphorothioate linkages by *in vitro* transcription using nucleoside α-phosphorthio triphosphates. T7 RNA polymerase specifically incorporates the Sp isomer of α-phosphorthiotriphosphate with inversion of configuration to produce the Rp isomer of the phosphorothioate linkage. The methods to produce transcripts fully substituted with phosphorothioate linkages adjacent to a given nucleotide, or to produce partially substituted transcripts containing approximately one phosphorothioate linkage per molecule, are described by Ruffner and Uhlenbeck (1990). Conrad *et al.* (1995) describe methods of using T7 RNA polymerase to produce chimeric transcripts containing ribonucleotides and

deoxyribonucleotides (with and without phosphorothioate linkages), and also ribonucleotides and 2'-O-methylnucleotides (with and without phosphorothioate linkages). These methods have been shown to produce transcripts containing up to 50% deoxyribonucleotides, and up to 58% 2'-O-methylnucleotides. Aurup *et al* (1992) describe methods for using T7 polymerase to produce transcripts containing 2'-fluoro-2'-deoxyuridine, 2'-fluoro-2'-deoxycytidine, and 2'-amino-2'deoxyuridine. (Aurup, 1992; Conrad, 1995; Ruffner, 1990) Further means for producing the nucleic acid molecules of this invention are further discussed below (Sambrook, 1989).

Nucleotides represented in the compounds above comprise a sugar, base, and a monophosphate group or a phosphodiester linkage. Accordingly, nucleotide derivatives or modifications may be made at the level of the sugar, base, monophosphate groupings or phosphodiester linkages. It is preferred that the nucleotides in the compounds above be oligonucleotides, ribonucleotides or RNA/DNA hybrids, however, other substitutions or modifications in the nucleotide are possible providing that ability to hybridise is not lost.

Nucleotide bases, deoxyribonucleotide bases, and ribonucleotide bases are well known in the art and are described, for example in Principles of Nucleic Acid Structure (Saenger, 1984). Furthermore, nucleotide, ribonucleotide, and deoxyribonucleotide derivatives, substitutions and/or modifications are well known in the art (See, for example, Saenger, 1984; Sober, 1970), and these may be incorporated in the nucleic acid molecule made with the proviso that the ability to hybridise to complementary nucleic acid sequences is not lost.

In addition, a large number of modified bases are found in nature, and a wide range of modified bases have been synthetically produced (See, for example, Saenger, 1984; Sober, 1970). For example, amino groups and ring nitrogens may be alkylated, such as alkylation of

ring nitrogen atoms or carbon atoms such as N1 and N7 of guanine and C5 of cytosine; substitution of keto by thioketo; saturation of carbon-carbon double bonds, and introduction of a C-glycosyl link in pseudouridine. Examples of thioketo derivatives are 6-mercaptopurine and 6-mercaptoguanine. Bases may be substituted with various groups, such as halogen, hydroxy, amine, alkyl, azido, nitro, phenyl and the like. The phosphate moiety of nucleotides or the phosphodiester linkages of oligonucleotides are also subject to derivatisation or modifications, which are well known in the art. For example, replacement of oxygen with nitrogen, sulphur or carbon gives phosphoramidates, (phosphorothioates, phosphorodithioates) and phosphonates, respectively. Substitutions of oxygen with nitrogen, sulphur or carbon derivatives may be made in bridging or non-bridging positions.

A further aspect of the invention provides alternative linkages such as an amide, carbamate, thiocarbamate, urea, amine, a sulfonamide, a hydroxylamine, a formacetal, a 3'-thioformacetal, a sulfide, allyl ether, allyl, ether, thioether, PNA (peptide nucleic acid) or an ethylene glycol function to replace the conventional phosphodiester linkage. These modifications may increase resistance towards cellular nucleases

Possible nucleotide modifications include sugar modifications such as 2' fluoro, 2' amino, 2' O-allyl, 2' C-allyl, 2' O-methyl, 2' O-alkyl, 4'-thio-ribose, arabinose, other sugars, or non-circular analogues.

Phosphate modifications may be phosphorothioate (non-bridging), phosphorodithioate (non bridging), 3' bridging phosphorothioate, 5' bridging phosphorothioate, phosphoramidates, 3' bridging phosphoramidate, 5' bridging phosphoramidate, methyl phosphonate, other alkyl phosphonates or phosphate triesters.

Modifications in base may be purine, 2,6-

diaminopurine, 2-aminopurine, O^6-methylguanosine, 5-alkenylpyrimidines, 5-propyne, inosine, 5-methylcytosine, pseudouridine, a-basic (ribose or deoxyribose).

Some nucleotides may be replaced with the following chemical linkers: 1,3-propane-diol, alkane-diols, or various polymers of (ethyleneglycol, tetraethylene glycol, hexaethyleneglycol).

Other Modifications to the 3' end may be selected from: 3'-3' inverted linkage (inverted diester or inverted phosphoramidate), 3'-3' linked abasic ribose, or end-capped (methoxyethylamine phosphoramidate).

Modified sugars may be synthesized as follows: 2'-deoxy-2'-fluoro uridine (Sinha, 1984); 2'-deoxy-2' fluoro cytidine (Sinha, 1984); 2'-deoxy-2' fluoroadenosine; synthesis and incorporation into nucleic acid molecule (Olsen, 1991); 2'-deoxy-2'-amino uridine and 2'-deoxy-2'-amino cytidine (Heidenreich, 1994); 2'-O-allyl-(uridine or cytidine or adenosine or guanosine) (Available from Boehringer Mannheim, Mannheim, Germany) or (Badger, 1994). 2'-deoxy-2'-C-allyl-ribonucleotides; 2'-O-methyl ribonucleotides see Review: (Sproat, B.S., 1991A) (also available from Chemgenes, Waltham, Mass. or Glen Research, Sterling, Va), other 2'-O-alkyl-ribonucleotides, synthesis see (Monia, B.P., 1993; Sproat, B.S., 1991B); α-anomer of uridine, cytidine, adenosine and guanosine, see (Debart, F., 1992 and references therein); other modified sugars, etc. Arabinose (Garbesi, A., 1993); Hexose-thymidine (Augustyns, K., 1992) and linear analogues of sugars (Hendry, 1994).

Modified phosphates may be synthesized as follows: Phosphorothioate; synthesized by modification of oxidation procedure during phosphoramidite synthesis. Reagents commercially available from Perkin Elmer and others, products are mixture of isomers, some methods available for stereospecific synthesis of phosphorothioate, see ref: (Stec, 1991); Phosphorodithioate; (Eldrup, A.B., 1994; Caruthers, 1991;

Beaton, 1991); 3'-bridging phosphorothioate; 5' bridging phosphorothioate; phosphoramidates (non-bridging, oxidize the phosphite triester with solution containing the required amine); (Froehler, B., 1988; Jager, A., 1988; Letsinger, R.L., 1988); 3' bridging phosphoramidate (NH replaces 3' O) (Forms very stable duplexes) (Letsinger, 1992; Gryaznov, S.M., 1995; Chen, J.K., 1995); 5' Bridging Phosphoramidate (NH replaces 5' O; thymidine analogue only, weak binder) (Gryaznov, S.M., 1992); Methylphosphonate (reagents are commercially available; Glen Research or Chemgenes Stereospecific; Rp isomers bind stronger: (Savchenko, 1994; Miller, 1991); 5'-deoxy, 5'-methylphosphonate (Szabo, 1995); Other alkyl-phosphonates (Fathi, 1994A; Fathi, 1994B); Phosphate triesters(Summers, 1986).

Replacements for the phosphodiester linkage may be synthesized as follows: For review see (De Mesmaker, 1995) Amides (Chur,1993; Blommers, 1994; De Mesmaeker, 1993; De Mesmaeker, 1994A; De Mesmaeker, 1994B; Lebreton, 1993; Lebreton, 1994A; Lebreton, 1994B; Idsiak, 1993): Carbamate (Waldner, 1994; Stirchak, 1987; Habus, 1994; Thiocarbamate (Waldner,1995); Ureas (Waldner, 1994) Amines (De Mesmaeker,1994C; Caulfield, 1994); Hydroxylamine (Debart,1992; Vasseur,1992; Formacetal (Matteucci,1990; Jones, 1993) Thioformacetal (Jones,1993); Allyl ether (Cao,1994); Allyl, Ether, Thioether (Cao, 1994); Alkane (De Mesmaeker,1994; PNA A selection of binding and antisense properties (Nielsen, 1993A; Hanvey, 1992; Egholm, 1993; Nielsen, 1993B) ; PNA Synthesis (Egholm, 1992A; Egholm, 1992B) ;Preparation of purine PNA monomers and oligonucleotides (available commercially from Millipore corporation).

Modified bases may be synthesized as follows: Purine; synthesis and incorporation into nucleic acid molecule (Slim, 1992; Fu, 1992; Fu, 1993); 7-deazaGuanosine, synthesis and incorporation into nucleic acid molecule (Fu, 1993); Inosine, synthesis and

incorporation into nucleic acid molecule (Slim,1992; Fu, 1993) 7-deazaAdenosine, synthesis and incorporation into nucleic acid molecule (Fu, 1992; Seela, 1993). O^6-methylguanosine, synthesis and incorporation into nucleic acid molecule (Grasby, 1993); 2,6-diaminopurine, synthesis (Sproat, 1991); 2-aminopurine, synthesis and incorporation into nucleic acid molecule (Ng, 1994; Tuschl, 1993); Isoguanosine, synthesis and incorporation into nucleic acid molecule (Ng, 1994; Tuschl, 1993); Xanthosine, synthesis and incorporation into nucleic acid molecule (Tuschl, 1993); 6-azathymidine, 6-aza-2'-deoxycytidine, synthesis and incorporation into oligonucleotides (Sanghvi, 1993); 5-alkenylpyrimidines; 5-propyne (Gilead, Froehler); inosine; 5-methylcytosine; pseudouridine; abasic ribose or deoxyribose.

As discussed above, once the nucleic acid molecules have been synthesised, or otherwise obtained, they may be immobilised onto the nanotube by a variety of methods including those methods normally used for coupling nucleic acids to solid supports. For references describing these methodologies, see Silman, I.H. and Katchalski, E. in Annual Review of Biochemistry, Vol. 35, p. 873 (1966); Melrose, G.J.H., in Review of Pure and Applied Chemistry, Vol. 21, p. 83, (1971); and Cuatrecasas, P. and Anfinsen, C.B., in Methods in Enzymology, Vol. 22, (1971). For example, nucleic acid molecules may be covalently or non-covalently associated with the carbon nanotubes of this invention.

Nucleic acid molecules can be attached to solid supports such as the surfaces of carbon nanotubes by diverse non-covalent interactions including simple non-covalent absorption driven by free energy changes of the system. However, pre-synthesised DNA molecules bound in this way lie flat against the surface of the nanotubes (Tsang, S.C. *et al.*, 1997, Angew. Chem. Int. Ed. Engl., 36,2198-2200) and the single strands of DNA are most likely are unable to hybridise to their complementary DNA

strands. Particularly advantageous procedures for physical attachment of nucleic acids to nanotubes, that will allow the physically-attached single-stranded nucleic acid to bind its complementary strand, involve firstly the physical absorption of a non-nucleotide or an oligonucleotide anchor to the nanotubes followed by chemical attachment of pre-made DNA to the anchor or build-up of the DNA from the physically bound anchor as described above.

Nucleic acid molecules can also be attached to solid supports such as carbon nanotubes by covalent coupling of the nucleic acid to the surface. Particularly advantageous procedures for chemical attachment of nucleic acids to nanotubes involve modifying the nanotubes by either direct functionalisation of nanotube tips or active sites on the nanotube walls, or attachment of a functional linker to the tips and/or the walls of the nanotubes. Both of these methods result in covalent attachment of the nucleic acids to the nanotubes. It will be appreciated that the type of initial treatment and type of attachment are partially dependent upon the end use of the nanotube.

The attachment of the nucleic acid molecule on the tips or at active sites on the walls of the nanotubes requires the nanotubes to be functionalised to produce a free carboxyl or hydroxyl group. The attachment of the nucleic acid molecule on the walls of the nanotubes i.e., along the sides or length of the nanotube requires the attachment of a linker, for example an azido compound, to the nanotube such that a free functional group such as hydroxyl, carboxyl, amine, hydrazine, aldehyde or a maleimide, can be provided for further growing the DNA *in situ* from that site or for chemical reaction with a pre-synthesised DNA molecule containing a functional group such as amine, carboxyl or sulfhydryl. The DNA molecule may be functionalised with these groups either on the 5′ or 3′ end, using 5′-modifier phosphoramidite or 3′-modified CPG available from Glen Research.

In the first method, nanotubes are functionalised by chemical treatment with nitric acid and sulphuric acid solution to produce hydroxyl and carboxyl functional groups (see for example: Liu *et al.*, Science 1998, 280, 1253; Chen *et al.*, Science 1998, 282, 95; Hamon *et al.*, Adv. Mater. 1999, 11, 834; Sloan *et al.*, Chem. Commun. 1998, 347.) For example, in a preferred methodology, the nanotube is refluxed in HNO_3 and/or H_2SO_4 to open the nanotube tips and to introduce -COOH and -OH groups at the open ends.

In a preferred embodiment, free hydroxyl groups are predominantly produced (together with carboxyl groups) using milder acid conditions. The nucleic acid(s) are then built up *in situ* from the hydroxyl groups using the automated DNA synthesizer. In a further preferred embodiment, the hydroxyl group can be reacted with functional group W on a difunctional spacer-molecule W-S-X of formula I as defined above, in order to extend the second functional group X further from the surface of the nanotube and thus allow for attachment of a DNA molecule to X with less steric hindrance from the nanotube. If X is a hydroxyl group, the nucleic acid molecules can be built up *in situ* using the automated DNA synthesizer (Scheme 1a). Alternatively, if X is a different functional group such as carboxyl or amine, a pre-synthesised DNA containing a linker with functional group Y can be attached to the extended functional groups on the nanotubes by reaction of Y with X using methods known in the art (Scheme 1b). For example, when X is a carboxyl group, Y may be an amine group. W-S-X may be, for example, succinic anhydride, or bromoacetic acid.

In another preferred embodiment, nanotubes modified with carboxyl groups are attached to the nucleic acid molecule using reaction conditions compatible with carboxyl groups. The nucleic acid molecule may be presynthesised with a linker containing a functional group, for example, an amino linker using any suitable

technique known in the art, and this may be covalently attached to the nanotubes via reaction of the amine group on the DNA with the carboxyl groups on the nanotubes to form an amide bond (scheme 2a). In a further preferred embodiment, the carboxyl group can be reacted with functional group W_a on a difunctional spacer-molecule W_a-S_a-X_a of formula Ia as defined above, in order to extend the second functional group X_a further from the surface of the nanotube and thus allow for attachment of a DNA molecule to X_a with less steric hindrance from the nanotube. If X_a is a hydroxyl group, the DNA can be built-up *in situ* using phosphoramidite chemistry (Scheme 2b). If X_a is some functional group other than hydroxyl, a pre-synthesised DNA containing functional group Y_a can be attached to the functional group X_a on the nanotube using methods known in the art. (Scheme 2a). If X_a is a carboxyl group, an amino-modified DNA can be used to react with this carboxyl group and form an amide bond, using any suitable known method, such as the hydroxy succinimide active ester method or the acid chloride method.

In the second method, a linker containing functional groups is attached to the walls of the nanotubes by a photochemical reaction. The linker is preferably an azido compound such as azido-thymidine or azidonitrobenzoyloxysuccinimide. It is attached by exposing the nanotube to the azido compound and then irradiating the exposed nanotube with UV light. The azido compound binds non-specifically to the nanotube and provides a free hydroxyl or carboxyl group.

In a preferred embodiment, the free hydroxyl groups on the 5′ position of the AZT provide sites from which nucleic acid molecules can be grown *in situ* using the automated DNA synthesizer (Scheme 3a). In a further preferred embodiment, the hydroxyl group can be reacted with functional group W on a difunctional spacer-molecule W-S-X of formula I as defined above, in order to extend the second functional group X further from the surface of

the nanotube and thus allow for attachment of a DNA molecule (Scheme 3b). If X is a carboxyl group, it may be reacted with the amine group of a pre-synthesised amino-modified nucleic acid molecule to form an amide bond using any suitable known method, such as the hydroxy succinimide active ester method or the acid chloride method.

In yet a further embodiment, the nanotubes are functionalised with linkers which are attached to the nanotubes by a photochemical reaction and which contain suitable functional groups including, carboxyl, or activated carboxyl, amine, isothiocyanate or maleimide, for example, azido nitrobenzoyloxysuccinimide, 4-(P-Azidosalicylamido) butylamine, Azidophenyl isothiocyanate, 4-(P-Azido salicylamido) butyl-3' (2'-pyridylthio)propionamide, 4-(P-Azido salicylamido) butyl-maleimide propionamide, and p-azidophenyl glyoxal monohydrate.

In a further embodiment, the nucleic acid molecule is then reacted with the modified nanotube using reaction conditions compatible with the functional group. This may require the nucleic acid molecule to be presynthesised so as to provide an amino linker or carboxyl linker or sulphydryl linker using any suitable known technique as described herein. For example, a nucleic acid molecule(s) having an amino linker can be covalently linked via an amide bond to nanotubes photoetched with azido nitrobenzoyloxysuccinimide or Azidophenyl isothiocyanate groups (Scheme 4a).

In yet a further preferred embodiment, the photoetched functional group is reacted with a difunctional spacer to produce an hydroxyl functional group extended from the surface of the nanotube. For example, the difunctional group may be 2-chloroethanol. The nucleic acid(s) are then built up *in situ* in an automated DNA synthesiser from the hydroxyl group on the spacer (Scheme 4b).

It is important that the amine group is free for

reaction in cases where amine-modified DNA molecules are to be reacted with carboxyl groups on nanotubes. When deprotecting the bases of the DNA molecule in ammonium hydroxide, the released protecting groups of adenine and cytosine bases are able to react with the primary amine to form an amide bond which renders the amine linker inactive. This inactivation can be avoided by treating the 5'-amine-modified DNA in ammonium hydroxide for a short time (20 minutes) at room temperature to release the molecule from the CPG (this short treatment at RT keeps the bases protected). The protecting group (MonoMethoxyTrityl, MMT) on the 5'-amine group can be removed by acid treatment, either before or after release of the molecule from the CPG. The Fmoc group on the 3'-amine group can be removed in a piperidine/DMF mixture for 2 h. Once the amine group on the base-protected DNA is reacted with the carboxyl groups on the nanotubes, the protecting groups on the bases can be removed by reacting in ammonium hydroxide for 8 hours at 55 °C.

Once the desired nucleic acid molecule or molecules are attached to the nanotube, these are capable of being used in a number of ways. For example, if an oligonucleotide is chemically or physically attached to a nanotube either aligned or in random orientation, it may be used to capture a target DNA strand, if the target is substantially complementary to the DNA immobilised on the nanotubes. Two nucleic acid sequences are "substantially complementary" when at least about 85%, preferably at least about 90%, and most preferably at least about 95%, of the nucleotides or ribonucleotides are able to form base-pair matches (adenine with thymine, guanine with cytosine) over the defined length of the nucleic acid sequences. Sequences that are substantially complementary can be identified in a hybridization experiment, for example under "stringent conditions" as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See e.g.,

Sambrook *et al.*, DNA Cloning, vols. I, II and III. Nucleic Acid Hybridization. However, ordinarily, "stringent conditions" for hybridization or annealing of nucleic acid molecules are those that

(1) employ low ionic strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium citrate/0.1% sodium dodecyl sulfate (SDS) at ~50°C (the exact temperature will depend on the number of base pairs, with the optimum temperature being 1-2°C below the melting temperature of the double helix which may be lower than 50°C for short double helices), or

(2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750mM NaCl, 75mM sodium citrate at 42°C.

Another example of "stringent conditions" is use of 50% formamide, 5 X SSC (0.75M NaCl, 0.075M sodium citrate), 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 X Denhardt's solution, sonicated salmon sperm DNA (50µg/mL), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 X SSC and 0.1% SDS.

The hybridisation event may be measured by a change in the electrical or electrochemical properties of a DNA sequence. Patterned or clustered nanotubes, each with a DNA of a different sequence chemically attached, may be used to screen for the presence of several target DNA molecules. The DNA may be immobilised either on the surfaces of the nanotubes and/or the tips. Devices with this configuration could be used as DNA biosensors, or as chips for DNA computers.

In general a biosensor is an analytical device that combines the specificity of a biological sensing element with a transducer to produce a signal proportional to target analyte concentration. Nanotubes with attached nucleic acid molecules act as a receptor in biosensing for the detection of complementary nucleic acid strands.

These biosensors would be useful in clinical applications, eg screening for the presence of bacterial or viral nucleic acids, in pharmaceutical applications, agricultural applications, food control, hygiene and environmental monitoring and forensic applications.

The signal detection can result from a change in mass, or reduction in conductivity, or from using electrochemical techniques such as cyclic voltammetry (Garnier *et al.* 1999, Synth. Met. 100, 89-94), chronopotentiometry (Wang *et al.* 1997, Biosensors & Bioelectronics, 12, 587-599), electrochemical impedence spectroscopy (Brett *et al.* 1999, Electrochim. Acta 44, 4233-4299) or by field effect transistor amplification (Souteyrand *et al.*, 1997, J. Phys. Chem. 101,2980-2985), photocurrent spectroscopy(Lassalle *et al*, 2001. Biosensors & Bioelectronics, 16, 295-303), from acoustic properties to be detected by piezoelectric quartz crystals (Ketterer *et al.*, 2000, Sensors & Actuators, b 65 (73-75), potentiometric amperometric, or optical transducer such as surface plasmon resonance (Bier *et al.* Sensors & Actuators, 1997, b 38-39 (78-82)), resonant mirror (Watts *et al.* 1995, Anal. Chem. 57, 4283-4289, Buckle, *et al.* 1993, Biosensors and Bioelectronics, 8, 355-363) or raman spectroscopy (Vo-Dinh *et al*, 1994, Anal. Chem., 66, 3379-3383).

The signals may be further amplified and processed like other biosensors, known in the art. Similar to other biosensors, DNA biosensors are usually in the form of electrodes, chips and crystals, and hence hybridization on a sensory surface is a solid phase reaction.

The requirements for an ideal detector include high specificity and high sensitivity using a protocol that can be completed in a relatively short time. Moreover, systems that can be miniaturised and automated offer a significant advantage over current technology, especially if detection is needed in the field.

The electrochemical methods of detecting hybridisation events use the principle of electrical circuit completion. It is well known that carbon nanotubes are conductors of electricity. Accordingly, nanotubes are capable of detecting minute changes in conductivity. A DNA modified carbon nanotube of the present invention will have a specific, measurable electrical conductivity profile. Once a hybridisation event has taken place the electrical conductivity profile will change. This change is capable of being detected. For example, International patent application No. WO02/03050 describes a system of detecting hybridisation using the completion of an electrical circuit. However, this patent application also describes the problems inherent in the detection of electrochemical changes. The conventional theory in the electrochemical methods is that it is essential for the reference electrode potential to be very stable and not be affected by chemical changes in the solution. By using carbon nanotubes these problems are avoided.

A further use of the invention described herein is the ability to place dispersed nanotubes in desired locations using the attached nucleic acid molecules as locators. In this case, DNA of complementary sequence to the DNA on the nanotubes is chemically or physically bound to the desired location by writing with DNA ink via pens such as AFM tips or ink-jet printers, or through patterning e.g. using photolithography. The nanotubes are then brought to these locations through DNA-DNA hybridisation. This configuration will have applications in the self-assembly of devices made from carbon nanotubes, including the self-assembly of electronic circuits and devices on the nanometer scale.

Nanotubes chemically or physically modified with DNA may be linked to other DNA-modified nanotubes through DNA-DNA hybridisation either directly via the DNA molecules attached to the nanotubes, or indirectly via

bridging DNA molecules with a variety of configurations. The nanotubes may be aligned roughly in parallel, when DNA on the walls of the nanotubes links the nanotubes side-by-side, or when the DNA on the tips of the nanotubes links nanotubes end-to-end. Devices formed by side-by-side linkages may be used as actuators when the nanotubes are laid on a non-expanding substrate. Devices formed by end-to-end linkages, or small bundles of nanotubes linked side-by-side and end-to-end, may be used as nano-scale conductors, more specifically wires in nano-electronic applications, as replacements for damaged nerves in prosthetic applications, or as the bio-electronic interface in bio-electronic devices. Alternatively, the nanotubes may be linked at angles to each other, including at right-angles to each other, when combinations of nanotubes are used with DNA modifications on the surfaces, and on the tips. Such configurations could have applications as transistors or gated devices.

Although the invention has been described with reference to presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Moreover, the following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated.

EXAMPLE 1: CHEMICAL AND PHYSICAL ATTACHMENT OF DNA TO CARBON NANOTUBES

Nucleic acid molecules have been covalently attached to carbon nanotubes using a number of different methods. The different strategies involved (1) aligned nanotubes or dispersed nanotubes, with different functional groups (hydroxyl or carboxyl) introduced predominantly on the tips of the nanotubes by chemical reaction, (2) aligned nanotubes, dispersed nanotubes, or mats of nanotubes, with functional groups introduced on to

the nanotubes by photochemical reaction , using photoraeactive functional groups such as azidothymidine, azido nitrobenzoyloxysuccinimide. In both strategies, the nucleic acid may be attached either by DNA synthesis *in situ* or by covalent coupling of pre-synthesised and functionalised DNA molecules to the nanotubes.

(1) *In situ* DNA Synthesis on Aligned, multi-walled Carbon Nanotubes with Functional OH/COOH Groups.

Aligned, MWNTs were grown on a quartz substrate, by pyrolysis of iron(II) phthalocyanine (FePc) under Ar/H_2 at 800-1100 °C (Li, D.-C., Dai, L., Huang, S., Mau, A.W.H. and Wang Z.L. (2000) Chem. Phys. Lett, 316, 349-355). The exposed ends of the nanotubes were sputtered with gold to form a thin gold-foil coating; this gold-foil coating with nanotubes was lifted off the quartz substrate to reveal clean ends of nanotubes free from amorphous carbon deposits. The tips of the nanotubes were functionalised with OH/COOH groups by refluxing the nanotubes in a mixture of $HNO_3/H_2SO_4/H_2O$ for 2-3 h. The nanotubes were washed with several changed of ultra-pure autoclaved water until the pH was >6. Nanotubes sitting on approximately 20-25 mm^2 of gold foil were then placed in the reaction column of an Applied Biosystems DNA synthesiser, and a DNA molecule of 16 nucleotides was built up on the nanotubes using the phosphoramidite method in accordance with the manufacturer's instructions. The base sequence of the DNA molecule synthesised on the nanotubes was as follows;

5'TAC GCG AAT TGC CAC T3'.

The DNA molecule was attached to the nanotubes at its 3' end. The progress of the reaction was monitored by the detritylation reaction at each step.

Approximately the same amount of nanotubes without any functional groups was used as a control to determine if the DNA was synthesised by covalent

attachment to nanotubes or just simply by physical adsorption of oligonucleotides on the nanotubes.

The DNA-modified nanotubes on gold foil, and the control nanotubes on gold foil, were transferred from the DNA-synthesiser column to a glass vial, and 1.4mL ammonia solution was added. The glass vial was sealed and then heated at 50-55°C for 8-10 hours. After cooling, the ammonia solution was removed, and the nanotubes were washed with several changes of ultra-pure, autoclaved water until the pH of the washes was <7. At no time in this procedure were the nanotubes allowed to become dry. The DNA-modified nanotubes were stored under water at 4°C.

To determine the amounts and nature of any DNA molecules that were in the supernatant, and hence not chemically bound to the nanotubes after the synthesis, the ammonia solution and washes were saved, combined, and then rotor-evaporated under vacuum to remove ammonia and concentrate the solution. The concentrated solution was treated with polynucleotide kinase and γ-[32]-P-ATP to attach a [32]-P-phosphate to the 5' ends of any DNA molecules present in the washes. The mix was electrophoresed on a 15% polyacrylamide gel containing 7M urea, and the pattern was visualised by exposure on a Molecular Dynamics PhosphorImager. Typically the pattern revealed DNA molecules varying in length from 1-2 nucleotides through to full-length, which indicated that some DNA molecules were being synthesised from nucleotides adsorbed on the surface of the nanotubes, and that some or all of these were removed on treatment with ammonia and/or by repeated washing. A small amount of full-length DNA, relative to the total amount of DNA, was in the supernatant taken from the oxidized nanotube sample, whereas a much higher proportion of full-length DNA, relative to the total amount of DNA, was in the supernatant taken from the control nanotube sample. The smaller amount of full-length DNA relative to the total DNA in the supernatant was suggestive of chemical

attachment of full-length DNA to the oxidized carbon nanotubes.

(2) Attachment of a Pre-Synthesised DNA to Carbon Nanotubes

DNA with amino linker was made on an Applied Biosystems DNA synthesiser.

The sequence of the 16-nucleotide DNA molecule was:

(5′ NH_2-$(CH_2)_6$- TAC GCG AAT TGC CAC T 3′) or

(5′ TAC GCG AAT TGC CAC T $(CH_2)_7$-NH_2 3′).

The DNA with the 5′-amino linker was treated with TCA/DCM for 5 min while on the DNA synthesizer to remove the MMT protecting group from the 5′-amino group. The DNA with the 3′-amino linker was treated with a 50/50 mixture of piperidine/DMF for 2 h at RT to remove the Fmoc protecting group from the 3′-amino group. These molecules were left to sit in 1mL of ammonia solution for 30 min at room temperature to release them from CPG beads. (2a) 2.1 mg of dispersed, oxidized, multi-walled nanotubes,refluxed in HNO_3) were suspended in 1.5 ml thionyl chloride and heated to 80°C for 3 h. The thionyl chloride was then decanted and the nanotubes were washed thoroughly with anhydrous acetonitrile. 40 nmole of 16-mer DNA with an amino hexane linker on the 5′ end and partially deprotected bases was dissolved in 1.5 ml of anhydrous DMF and added to the acid chloride modified nanotube. 5 μl of DIEA was added to the reaction mixture to adjust the pH=9. The suspension mixture was shaken gently under nitrogen atmosphere at RT for 16 h. The DMF solution was decanted after centrifugation of the reaction mixture and precipitation of the nanotubes. The nanotubes were partly suspended very uniformly in DMF and most did not precipitate even at high-speed centrifugation.

The DNA-modified MWNTS were transferred with water to a glass vial. Since it was difficult to pellet the nanotubes, the following procedure was used when changing solutions. The nanotubes were centrifuged at 12,000 rpm for ~10 minutes. Most of the water was removed, and then the nanotubes were washed with 700μL ammonia solution. The nanotubes were again centrifuged at 12,000 rpm for ~10 minutes. Most of this ammonia solution was removed, and replaced with 1.4mL of fresh ammonia solution. The glass vial was sealed, and heated at 55°C for 8-10 hours. The black-brown suspension, which formed above the nanotubes in the solid phase, was removed, and saved. The nanotubes were washed with several changes of ultra-pure, autoclaved water until the pH of the washes was <7. At no time in this procedure were the nanotubes allowed to become dry. The DNA-modified nanotubes were stored under water at 4°C.

Sequences of DNA molecules attached to carbon nanotubes were:

NT3'-$NH_2$3': 5' TACGCGAATTGCCACT-$(CH_2)_7$-NH_2 3' + COOH-NANOTUBES (attachment of 3' end of DNA to nanotubes) and

5'NH_2-NT3': 5' H_2N-$(CH_2)_6$-TACGCGAATTGCCACT 3' + COOH-NANOTUBES (attachment of 5' end of DNA to nanotubes).

(2b) 2.1 mg of dispersed, oxidized SWNTs (Iljin)which was refluxed in HNO_3 for 3 hr, filtered, washed thoroughly with water and dried, was suspended in 0.6 ml DMF. 10 mg DiSuccinimideCarbonate (DSC) dissolved in 0.4 ml DMF was added to the nanotube suspension and sonicated for 1.5 h while shaken gently under nitrogen atmosphere. The black/brown suspension was precipitated by centrifuging the mixture at 14000 rpm for 30 min. 0.5 ml fresh DMF was added to the precipitate, and the mixture was vortexed for 1 min and then centrifuged at 14000 rpm for extra 30 min. A 16-mer DNA with an amino linker on the 3' end

synthesized by automated DNA synthesis using (Dimethoxytrityloxy-3 fluorenmethoxycarbonylamino-hexane-2-methoxysuccinoyl) long chain alkyl amino-CPG) was released from CPG by treatment in ammonia solution for 20 minutes.

Sequence of DNA molecules attached to carbon nanotubes was:

NT3'-$NH_2$3': 5' TACGCGAATTGCCACT-$(CH_2)_7$-NH_2 3'

25 nmol of this DNA with partially deprotected bases was dissolved in 0.5ml of anhydrous DMF and added to the succinylated modified nanotube. 5 μl of DIEA was added to the reaction mixture to adjust the pH=9. The suspension mixture was shaken gently under nitrogen atmosphere at RT for 16 hr. The sample was more suspended in the DMF after the reaction. Centrifugation did not precipitate the whole nanotube sample. DMF was evaporated off and the water was added to the precipitate. A black suspension was formed, indicative of the attachment of DNA to the nanotubes. The water was removed by rotary evaporation and the DNA was treated in ammonium hyroxide solution at 55 °C for 8 h. The ammonium hydroxide was removed and the nanotubes were washed three times with water. The samples were store at 4 °C under water.

(3) Photoetching of AZT on the surface of the nanotubes followed by *in situ* DNA Synthesis.

About 10-50 µg of azidothymidine (AZT) (dissolved in ETOH) was coated on to aligned, multi-walled nanotubes attached to a piece of gold foil of area about 25 mm^2. The solution was allowed to evaporate at room temperature. The samples were air-dried and then irradiated with 450W medium pressure lamp with max output at around 254 nm for 5 mins (112 mW/cm^2). The samples were then washed thoroughly with ETOH to remove all the unreacted AZT. The

removal of the unreacted samples was assessed by monitoring the wash solution by HPLC.

The AZT photoetched nanotubes were then placed on a DNA synthesiser as described above and a 16-nucleotide and a 35-nucleotide DNA molecule was synthesised by the phosphoramidite method. As described above, the blocking groups on the synthesised DNA were then removed with ammonia solution.

The DNA molecules were attached to the nanotubes through their 3' ends. Sequences of DNA molecules attached to carbon nanotubes were:

NT3'(azidoT): 5' TACGCGAATTGCCAC(azidoT)-

NT3'T_{19}(azidoT): 5' TACGCGAATTGCCAC$(T)_{19}$(azidoT)

(4) Photoetching of a linker on the surface of the nanotubes followed by attachment of a Pre-Synthesised DNA containing a functional group.

About 10-50 μg of azidobenzoyloxysuccinimide (dissolved in DCM) was coated on to aligned, multi-walled nanotubes attached to a piece of gold foil of area about 25 mm^2 or to a mat of unmodified SWNTs formed by filtration of a suspension of the SWNTs in DMF. The zidobenzoyloxysuccinimide solution was allowed to evaporate at room temperature. The samples were air-dried and then irradiated with 450W medium pressure lamp with max output at around 254 nm for 5 mins (112 mW/cm^2) . The samples were then washed thoroughly with DCM to remove all the unreacted azido-linker. The photoetched nanotubes were then reacted with 5'-amino modified DNA (pre-treated with piperidine/DMF to remove the Fmoc group from the 5' amine group and then deprotected partially for 20 min at RT in ammonia solution to release the DNA from the CPG). The reaction was carried out in a DMF solution for 16 h and the pH was adjusted to 9 by adding DIEA. After coupling, the supernatant was removed and the nanotubes

were washed several times with DMF and water. The blocking groups on DNA were then removed by exposure to ammonia solution at 55 °C for 8 h.

The Sequence of the DNA molecule attached to carbon nanotubes was:

5' NH_2-$(CH_2)_6$-TACGCGAATTGCCAC

(5) Physical attachment of DNA to carbon nanotubes - *In situ* DNA Synthesis on Aligned Multi-Walled Nanotubes.

Unmodified, aligned, multi-walled carbon nanotubes on a piece of gold foil approximately 20 mm^2 in area were placed in a reaction chamber on an Applied Biosystems DNA synthesizer. As a control, a similar amount of gold foil containing aligned, multi-walled carbon nanotubes, modified with azidothymidine by a photochemical reaction as described above, were placed in a second reaction chamber.

A 35-nucleotide DNA molecule (NT3'-T19) was synthesised in each reaction chamber by the phosphoramidite method. As a further control for the physical attachment, a 16-nucleotide DNA molecule (NT3') was synthesized in an additional reaction chamber. As described above, the blocking groups on the synthesised DNA were then removed with ammonia solution.

Sequences of DNA molecules physically attached to carbon nanotubes were:

5' TACGCGAATTGCCACTTTTTTTTTTTTTTTTT 3' and
5' TACGCGAATTGCCAC 3'

Sequence of DNA molecules chemically attached to the azidothymidine-modified nanotubes was:

5' TACGCGAATTGCCACTTTTTTTTTTTTTTTTT(azidoT).

After deblocking, the supernatant ammonia solution and

subsequent rinses with water were pooled, rotor-evaporated to dryness under vacuum, redissolved in water, labeled with P^{32}, electrophoresed on a 15% polyacrylamide gel containing 7M urea, and imaged on a Molecular Dynamics PhosphorImager. The image revealed that DNA ranging in length from 1-2 nucleotides to full-length material was in the ammonia solution and/or water rinses from both reaction chambers. This implies that DNA was synthesized in each reaction vessel by physical absorption on to the nanotubes, and that some of this physically adsorbed DNA, if not all, could be removed by washing. Tests to determine if any physically adsorbed DNA remained attached to the nanotubes, and to see if it was in a state where it could bind its complementary strand, are described below.

EXAMPLE 2: SYNTHESIS AND MANIPULATION OF DNA MOLECULES FOR ATTACHING TO THE NANOTUBES AND FOR USE IN ASSAYS TO DETERMINE THE STATUS OF DNA ATTACHED TO NANOTUBES.

DNA molecules were synthesised on an Applied Biosystems DNA synthesiser using the phosphoramidite method. The CPG-beads, with DNA attached, were tipped from the DNA-synthesiser columns into a glass vial, and 1-1.4mL of ammonia solution was added. The glass vial was sealed, and heated at 55°C for 8-10 hours. After cooling, the solution was transferred to a round-bottom flask, rotor-evaporated under vacuum to remove ammonia, and co-evaporated twice with autoclaved, milliQ water. The DNA was transferred with autoclaved water to an eppendorf tube, concentrated with sec-butanol, washed with ether, and pelleted on dry ice using 3M sodium acetate and four volumes of ethanol. The pellet was washed with 80% ethanol, dried under vacuum, redissolved in water, and stored frozen at -20°C.

The purity of each DNA oligomer was checked by labelling the 5′ end with [32]-P, using γ-ATP-[32]P and polynucleotide kinase, and electrophoresing the material

on a 15% polyacrylamide gel containing 7M urea. If the full-length DNA oligomer was less than 98% pure, the oligomer was purified by electrophoresis on a preparative polyacrylamide gel containing 7M urea; gel slices containing the full-length molecule were excised, crushed, and soaked in water for 24 hours. The supernatant containing DNA was extracted, concentrated with sec-butanol, washed with ether, extracted twice with phenol-chloroform, washed with ether, and pelleted on dry ice using 3M sodium acetate and four volumes of ethanol. The pellet was washed with 80% ethanol, dried under vacuum, redissolved in autoclaved, milliQ water, and stored frozen at -20°C.

Concentrations of DNA oligomers were determined by measuring the absorbance at 260nm and molar extinction coefficients (L. $Mole^{-1}$ cm^{-1}) of 15400 for Adenosine, 11700 for Guanosine, 7300 for Cytidine, and 8800 for Thymidine.

Sequences of functionalised DNA molecules synthesized for covalent attachment to nanotubes:

5'NH2-NT3': 5' NH_2-$(CH_2)_6$-TACGCGAATTGCCAC

NT3'-NH23': 5' TAC GCG AAT TGC CAC T $(CH_2)_7$-NH_2 3'

NT3'-T19'NH23': 5' TAC GCG AAT TGC CAC $(T)_{19}$ $(CH_2)_7$-NH_2 3'

Sequences of DNA molecules used for assays were:

NT3': 5' TACGCGAATTGCCACT 3'

NT3'antisense: 5' AGTGGCAATTCGCGTA 3'

NTbridge3': 5' AGTGGCAATTCGCGTACGGGGCCCCG 3'

Gold2A-SH3': 5' AGTGGCAATTCGCGTA-$(CH_2)_3$-S-[S-$(CH_2)_3$-OH] 3'

NTbridge5': 5' CGGGGCCCCGAGTGGCAATTCGCGTA 3'

Gold3A-SH3': 5' AGTGGCAATTCGCGTA$(T)_{19}$-$(CH_2)_3$-S-[S-$(CH_2)_3$-OH] 3'

EXAMPLE 3: DETERMINATION OF DNA ATTACHMENT ONTO NANOTUBES

X-ray Photoelectron Spectroscopy (XPS) assays the chemical composition of a 10nm surface layer. Thus,

XPS can be used to determine if DNA is attached to the surface of nanotubes, particularly by detecting the presence of phosphorus, and increased amounts of nitrogen. However, it is not sufficiently sensitive to distinguish between DNA that is chemically attached or strongly physically adsorbed, nor can it reveal if the attached DNA is in a conformation that is able to bind its complementary strand. In order to characterise the DNA-modified nanotubes, XPS (Kratos Ultra Imaging XPS spectrometer, Mg ka at 150 W) was carried out. The results of this analysis are shown in Table 1-3. The data shown in Table 1 are for the samples prepared in Example 1(1). The data shown in Table 2 are for the samples prepared in Example 1(2) for the 5'-amino modified DNA. The data shown in Table 3 are for the samples prepared in Example 1(3). XPS analysis of nanotubes photoetched with AZT for various times.

To investigate further the extent of photochemical linkage with changing the irradiation time, XPS analysis of unmodified nanotubes was compared to nanotube samples dosed with the same amount of AZT and photoirradiated with a 450 medium pressure mercury lamp (20 mW/cm^2) for two different times, 3 min and 9 min (Table 4, "fully treated" sample, which produces DNA chemically attached to the nanotubes, was prepared as in Example 1(3), samples B and D, which produce DNA physically adsorbed to the nanotubes, were prepared as in Example 1(5)).

As shown in Tables 1-3, in all except one case, the percentage of O has increased and the percentage of C has decreased compared to the parent unmodified nanotube sample, as might be expected for DNA attachment to nanotubes; however the oxygen content of the unmodified nanotubes is unexpectedly high, and not constant, and so relative numbers of %P and %N are most likely to be more indicative than absolute numbers for % P, N, O, and C. In all methods used for attaching DNA to the nanotubes, the

percentage of N and P has increased compared to the parent unmodified nanotube sample, as would be expected for DNA attachment to nanotubes.

As shown in Table 3, the %N and %P has increased in both control samples B and D, relative to the unmodified sample A, which is indicative of DNA physically adsorbed on the surface of these nanotubes. However, the fully-treated sample which should produce chemical attachment of DNA to the nanotubes has even higher %P and %N than samples B and D, indicative of chemical attachment and, possibly, some physical adsorption, to the nanotubes.

As shown in Table 4, the percentage of nitrogen element on the surface of the nanotubes has increased with increased irradiation time, which is consistent with the increase of C-N bond energy at 285.97 ev. These two results provide evidence for increasing amounts of AZT chemically attached to the surface of the nanotube with increased irradiation time. The increase of C=O bond energy at 287.97eV is also consistent with carbonyl groups on the thymine base (on AZT) being attached to the surface.

TABLE 1

Aligned multi-walled Nanotubes with Hydroxyl groups (numbers show % of each element)

	Non-treated	DNA-attached
C^{1s}	76.89	73.5
N^{1s}	3.18	5.79
P^{2p}	-	0.83
O^{1s}	15.32	14.85

TABLE 2

Disp rs d, oxidised carbon nanotub s with Carb xyl groups (numbers show % of ach el ment)

	Non-treated	DNA-attached
C^{1s}	73.03	52.66
N^{1s}	2.41	3.90
P^{2p}	-	0.83
O^{1s}	23.5	35.24

TABLE 3

Aligned, multi-walled carbon nanotubes photoetched with AZT (numbers show % of each element)

	Control A	Control B	Control C	Control D	Fully treated
C^{1s}	91.86	86.09	76.97	83.16	79.96
N^{1s}	3.43	5.46	2.34	5.68	7.08
P^{2p}	0.02	0.70	-	0.76	1.07
O^{1s}	3.68	6.81	20.36	9.74	10.31

- A: No treatment
- B: No treatment, DNA synthesis
- C: AZT added, washed off, no synthesis
- D: AZT added, washed off, no irradiation, DNA synthesis
- Fully treated: AZT added, irradiated, DNA synthesis

TABLE 4

Aligned multi-walled carbon nanotubes photetched with AZT for varying times (numbers show % of each element)

	Control	Sample A	Sample B
C^{1s}	84.34	90.49	88.89
N^{1s}	1.20	1.87	3.13
O^{1s}	14.24	7.64	7.98
C^{1s}(285.97 ev, C-N)	3.62	4.98	5.41
C^{1s}(286.57 ev, C-O)	5.43	7.60	5.22
C^{1s}(287.97ev, C=O)	2.76	3.53	4.57

Control: Aligned MWNts with no treatment
Sample A: Aligned MWNts treated with AZT and exposed to 3 min irradiation
Sample B: Aligned MWNts treated with AZT and exposed to 9 min irradiation

EXAMPLE 3: ASSAYS TO QUANTIFY AMOUNTS, AND LOCATIONS, OF DNA ATTACHED TO THE NANOTUBES AND TO DETERMINE IF THE ATTACHED DNA IS IN A FUNCTIONAL STATE FOR HYBRIDIZATION.

It is possible that a DNA molecule that is chemically attached at one end to carbon nanotubes may be lying strongly adsorbed on the nanotubes at its "free" end, and hence not be in a conformation that is able to bind to its complementary DNA strand when this is added. We have developed two assays to determine if the chemically attached DNA is in a useful state. The ^{32}P assay is easier to perform, and also produces quantitative data on the number of DNA molecules of complementary sequence which are hybridized to the DNA molecules attached to the nanotubes. The gold-nanoparticle assay is a striking visual assay that reveals the sites of attachment of the chemically-bound DNA molecules, and it also produces a minimal estimate of the numbers of DNA molecules that have bound their complementary strands.

^{32}P Assay

This assay tests if the DNA, which is chemically attached to the carbon nanotubes, is able to hybridise to its complementary strand and also it is able to distinguish its complementary strand from other DNA molecules.

Complementary, and non-complementary, DNA molecules, with radioactive (^{32}P) phosphate groups attached to their 5′ ends, are added to the DNA-modified nanotubes, and unmodified nanotubes as controls. The amount of radioactivity remaining on the nanotubes after extensive washing permits the determination of the numbers of added DNA molecules which are bound to DNA molecules chemically attached to the carbon nanotubes, with corrections for non-specific binding.

Attaching the radioactive label to the DNA. 80pmol of DNA oligomer were added to 1μl polynucleotide

kinase and 4µl γ-[^{32}P]-ATP, in a total volume of 20µl kinase buffer, and the mixture was allowed to react for 30-45 minutes at 37°C. The reaction was stopped with EDTA. The mixture was extracted twice with phenol-chloroform, washed once with ether, and then the DNA was pelleted from 0.3M sodium acetate, 80% ethanol, on dry ice. The DNA pellet was washed with cold 80% ethanol, dried under vacuum, dissolved in 80µl autoclaved milliQ water to form a 1µM solution and then stored frozen at -20°C. The base sequence of DNA molecules chemically attached to carbon nanotubes by the azidothymidine method was either:

5' TACGCGAATTGCCACT (NT3') or

5' TACGCGAATTGCCACTTTTTTTTTTTTTTTTTTT (NT3'-T_{19}).

The base sequences of DNA molecules ^{32}P-labelled on their 5' ends were:

5' AGTGGCAATTCGCGTA (NT3'AS, the antisense strand. NT3'AS has the base sequence which is complementary to the DNA attached to the nanotubes, and so it should bind to the DNA on the nanotubes if the latter is in a functional state).

5' TACGCGAATTGCCACT (NT3', the sense strand. NT3' has the same base sequence as the DNA attached to the nanotubes, and so it should not bind to the DNA on the nanotubes.)

Experiment A. Determining the extent of specific and non-specific binding of 5'-end ^{32}P -labelled DNA to DNA-modified multi-walled, aligned carbon nanotubes.

Samples 1 and 2 are aligned MWNT on gold foil, to which DNA (NT3'-T_{19}) is chemically attached by the azidothymidine method. Samples 3 and 4 are unmodified, aligned MWNT on gold foil. 5'-end ^{32}P-labelled DNA molecules (NT3'AS, or NT3') were added to the nanotube samples as indicated below.

	NT3'AS	NT3'
Nanotubes-T_{19}-NT3'	sample 1	sample 2
Nanotubes	sample 3	sample 4

Method: The DNA-modified carbon nanotubes were stored under sterile water at 4°C. Prior to starting the experiment, all samples of nanotubes were soaked in several changes of sterile water at 37°C.

Water on all nanotube samples was replaced over a period of 1 hour with 3 changes of 50-100µl 2X SSC, 0.1% SDS at 37°C. Following this equilibration, 5µl of 1µM ^{32}P-labelled DNA solution was added (NT3'AS to samples 1 and 3, and NT3' to samples 2 and 4), and the samples were gently rocked for 5 hours at 37°C. The supernatant containing unbound ^{32}P-labelled DNA was removed, and the samples were washed with 2X SSC, 0.1% SDS, until no counts could be detected in the washings (4-6 washings).

The samples were transferred on to a glass plate and were covered with plastic film (Saran wrap). The samples were exposed to a PhosphorImager screen, and counts were quantified on a Typhoon 8600 Variable Mode Imager (Molecular Dynamics) using Image Quant software and Storage Phosphor mode.

To account for differential ^{32}P labeling of NT3' and NT3'AS, the counts per NT3' and NT3'AS molecules were quantified on the Phosphor Imager from stock solutions with known concentrations. The area of each nanotube sample was measured under an optical microscope. Sample sizes varied from 4 to 8 mm^2. The total counts for each sample were then converted to nanomoles of ^{32}P-labelled DNA per mm^2, and are given in Table 5. Note that some of the edges of the nanotube samples were jagged, and so the measured areas are estimates. However, the numbers in Table 5 are sufficiently accurate to reveal trends.

Experiment B. Determining the effect of Express Hyb on

the binding of 5'-end ^{32}P -labelled DNA to DNA-modified multi-walled, aligned carbon nanotubes.

Samples 1 and 2 are aligned MWNT on gold foil, to which DNA (NT3') is chemically attached by the azido Thymidine method. Samples 3 and 4 are unmodified, aligned MWNT on gold foil. 5'-end ^{32}P-labelled DNA molecules were added to the nanotube samples as indicated below.

	NT3'AS	NT3'
Nanotubes-NT3'	sample 1	sample 2
Nanotubes	sample 3	sample 4

Method: The DNA-modified carbon nanotubes were stored under sterile water at 4°C. Prior to starting the experiment, all samples of nanotubes were soaked in several changes of sterile water at 37°C.

Water was removed from all carbon nanotube samples, and then ~50µl ExpressHyb (hybridization solution from Clontech (Palo Alto, CA)) were added to all samples. The samples were left to equilibrate with gentle rocking for 2 hours at 37°C; the ExpressHyb was replaced twice with fresh solution during this period. Then 5µl of 1µM ^{32}P-labelled DNA solution was added (NT3'AS to samples 1 and 3, and NT3' to samples 2 and 4), and the samples were gently rocked for 7 hours at 37°C. Unreacted ^{32}P-labelled DNA solution and ExpressHyb was removed, and the samples were washed 4 times with 2X SSC, 0.1% SDS, at which point no counts could be detected in the washings. The samples were transferred on to a glass plate and were covered with plastic film (Saran wrap). The samples were exposed to a PhosphorImager screen, and counts were quantified on a Typhoon 8600 Variable Mode Imager (Molecular Dynamics) using Image Quant software and Storage Phosphor mode.

To account for differential ^{32}P labeling of NT3' and NT3'AS, the counts per NT3' and NT3'AS molecule were quantified on the Phosphor Imager from stock solutions with known concentrations. The area of each nanotube

sample was measured under an optical microscope. Sample sizes varied from 7 to 11 mm^2. The total counts for each sample were then converted to nanomoles of ^{32}P-labelled DNA per mm^2, and are given in Table 5 below. Note that some of the edges of the nanotube samples were jagged, and so the measured areas are estimates. However, the numbers in Table 5 are sufficiently accurate to reveal trends.

TABLE 5

Nanomoles/mm^2 of ^{32}P-labelled DNA (either NT3'AS or NT3') remaining on aligned MWNT after hybridization and washing.

	MWNT-DNA + NT3'AS	MWNT-DNA + NT3'	MWNT + NT3'AS	MWNT + NT3'
Experiment A (no ExpressHyb)	172	95	138	193
Experiment B (with ExpressHyb)	90	6	58	29

The two columns on the right hand side of Table 5 show the extent of physical adsorption of DNA on unmodified MWNT. The presence of Express Hyb, as in Experiment B, reduces considerably the physical adsorption of DNA on unmodified nanotubes (138 compared with 58, 193 compared with 29).

Data in the last column (MWNT + NT3') compared with those in the second column (MWNT-DNA + NT3', where the attached NT3' on the nanotubes should not hybridise to the added NT3') indicate that physical adsorption is significantly reduced when the nanotubes are modified with DNA (193 compared with 95, and 29 compared with 6), and that this is further reduced, substantially, when Express Hyb is also present (95 compared with 6, column 2).

Thus, there is very little physical adsorption, or non-specific binding, of DNA molecules to DNA-modified nanotubes in the presence of Express Hyb.

Hence, data in the first column of Table 5 indicate that NT3'AS is binding specifically to the DNA (NT3'T19 or NT3') which is chemically attached to the nanotubes. This specific binding is further enhanced, relative to the low remaining level of non-specific binding, when Express Hyb is present (compare column 1 (90) and column 2 (6) in Experiment B).

Thus, specific binding of the DNA target strand to its complementary DNA strand which is chemically attached to carbon nanotubes is ~15-fold higher than the level of non-specific binding, in the hybridizing conditions used here (i.e. in the presence of Express Hyb).

Visual assay using DNA-modified gold nanoparticles and Transmissiom Electron Microscopy (TEM)

We have several means of determining if DNA is attached to carbon nanotubes, and if that DNA is in a functional state. For example, analysis by XPS indicates if the DNA is present on the surface of the nanotubes

either by physical adsorption or chemical attachment. Analysis with radioactively-labeled DNA, with a base sequence complementary to that of the DNA strands bound to the nanotubes, indicates if the bound DNA is able to bind its partner strand and how much is bound.

It would also be highly useful if we could see exactly where the DNA is chemically attached on the nanotubes, since for some purposes we would like DNA to be on the tips of the nanotubes (e.g. for end-to-end linkages) and for others we would like DNA to be on the sides (e.g. for maximum DNA loading for sensors, or for side-by-side linkages of nanotubes). Therefore we have developed a visual assay using gold nanoparticles. For this assay, DNA molecules, with base sequence complementary to those attached to the nanotubes, are synthesized with a terminal linker containing a di-thiol group, through which chemisorptionto gold nanoparticles occurs. The DNA strands on these modified gold nanoparticles bind to their partner DNA strands on the nanotubes, and thus bring the gold nanoparticles very close to the site of DNA attachment on the nanotubes. The locations of the gold nanoparticles are readily imaged by TEM.

In the following experiments, the base sequence of DNA molecules bound through the dithiol group to gold nanoparticles is either

5′ AGTGGCAATTCGCGTA-$(CH_2)_3$-S-[S-$CH_2)_3$-OH] 3′ (Gold2A-SH3′) or

5′ AGTGGCAATTCGCGTA$(T)_{19}$-$(CH_2)_3$-S-[S-$CH_2)_3$-OH] 3′ (Gold3A-SH3′).

The base sequence of DNA molecules chemically attached through their 3′ ends to carbon nanotubes is either

5′ TACGCGAATTGCCACT-nanotube (NT3′) or

5′ TACGCGAATTGCCACT$(T)_{19}$-nanotube (NT3′-T_{19}) or

5′ TACGCGAATTGCCACT-$(CH_2)_6$-NH_2 (NT3′-$NH_2$3′).

The base sequence of Gold2A-SH3' is complementary to that of NT3' and NT3'-$NH_2$3', and to the 16 nucleotides at the 5' end of NT3'-T_{19}. The base sequence of the 16 nucleotides at the 5' end of Gold3A-SH3' is complementary to that of NT3' and NT3'-$NH_2$3', and to the 16 nucleotides at the 5' end of NT3'-T_{19}.

The following procedure prepares gold nanoparticles of diameter ~ 14nm.

All glassware (500ml conical flask and 250ml storage bottle) and Teflon stirrer are first washed in aqua regia ($HCl:HNO_3$ = 3:1 by volume), and rinsed very well with milliQ water.

200ml of milliQ water in a clean 500ml conical flask is heated and gently stirred with a clean Teflon magnetic stirrer. While the water is being stirred and heated, 2ml of 1% (W/V) gold chloride solution is added. When the solution is boiling vigorously, 5ml of 1% (W/V) sodium nitrate is added as quickly as possible. The solution turns from pale yellow to colourless. The heat is reduced, but the solution is kept on the boil. Over a period of several minutes, the solution changes colour from clear to dark blue, to purple, to red. Once the solution is red, the heat is further reduced and the solution is kept boiling for about 10 minutes.

The solution is removed from the heat, and allowed to cool. A cover over the opening of the conical flask prevents dust contamination.

The average diameter and the size distribution of the gold nanoparticles is determined by imaging using a Transmission Electron Microscope. Typically, the diameter is approximately 14(±1) nm.

Determining the concentration of a solution of gold nanoparticles

Extinction coefficient (at 520nm) = $2.4 \times 10^8\ M^{-1}cm^{-1}$

Concentration (M) = $A_{(\sim 520nm)}$/extinction coefficient

Typically, the undiluted sample of a solution of gold nanoparticles, made by the above procedure, has an

absorbance maximum of ~1.2 at 519nm. So, a typical concentration is ~5nM.

Attachment of single-stranded DNA to gold nanoparticles

Small-scale preparation (to make ~700µl of 17nM DNA-modified gold nanoparticle solution)

This procedure essentially follows that described by Storhoff, J.J., Elghanian, R., Mucic, R.C., Mirkin, C.A., and Letsinger, R.L. (1998) J. Am. Chem. Soc. 120, 1959-1964.

2.2ml of a 5.0nM solution of gold nanoparticles were mixed with 9680 pmol (40µl of a 242µM solution) of Gold2A-SH3' in a Teflon tube (Nalge). The tube was wrapped in aluminium foil to keep out light.

The solution in the Teflon tube was rocked very gently overnight (~19-22 hours). At this stage the colour of the solution is red. Then, 26µl of 1M sodium phosphate buffer (pH 7.0) and 260 µl 1.0M NaCl solution were added to the DNA and gold nanoparticle solution in the Teflon tube (to make approximate final concentrations of 10mM buffer and 0.1M NaCl), and the solution was again gently mixed for 48 hours.

The solution was centrifuged at 12,000 rpm for 30 minutes. The colourless supernatant was removed leaving a small volume of dark-red solution containing the DNA and gold nanoparticles. This dark red solution was washed with 1.6ml of 0.1M NaCl, 10mM phosphate buffer, and centrifuged at 12,000 rpm for 30 minutes. Again the colourless supernatant was removed. The washing, centrifuging, and removal of supernatant was repeated.

640µl of 0.3M NaCl, 10mM phosphate buffer, 0.01% azide was added to the dark red solution to make ~17nM solution of DNA-modified gold nanoparticles.

Large-scale preparation (to make ~3ml of 17nM gold nanoparticle solution where the gold nanoparticles are modified with varying quantities of DNA)

10ml of 5nM gold nanoparticles are mixed with:

17593 pmol (7.27µl of 2.42mM Gold2A-SH3'), DNA strands :Au particles = 352 or

44044 pmol (18.2µl of 2.42mM Gold2A-SH3'), DNA strands :Au particles = 880 or

88088 pmol (36.4µl of 2.42mM Gold2A-SH3'), DNA strands :Au particles = 1762.

After gently mixing overnight, 110µl 1M sodium phosphate buffer and 1100µl 1M NaCl are added to the solution and the mixture is gently rocked for 48 hours. The solution was centrifuged at 12,000 rpm for 30 minutes. The colourless supernatant was removed leaving a small volume of dark-red solution containing the DNA and gold nanoparticles. This dark red solution was washed with 10ml of 0.1M NaCl, 10mM phosphate buffer, and centrifuged at 12,000 rpm for 30 minutes. The colourless supernatant was removed, and the washing, centrifuging, and removal of supernatant was repeated. The ~0.3ml of dark red DNA-modified gold solution was adjusted to ~3ml with 2.7ml of 0.3M NaCl, 10mM phosphate buffer, 0.01% azide solution to form a ~17nM Au (DNA modified) nanoparticle solution.

Visual Assay

This assay tests if the DNA which is attached to the carbon nanotubes is:

(i) able to hybridise to its complementary strand

(ii) able to distinguish its complementary strand from other DNA molecules; and

(iii) shows where the chemically attached DNA is located on the nanotubes.

A schematic drawing of gold nanoparticles with single-stranded DNA molecules attached is shown in Figure 1, and TEM images of gold nanoparticles functionalised with DNA is shown in Figure 2.

Complementary, and non-complementary, DNA molecules, bound to gold nanoparticles through dithiol groups on their 3' ends, are added to the DNA-modified nanotubes, and unmodified nanotubes as controls. The

locations of the gold nanoparticles on the nanotubes are visualized using Transmission Electron Microscopy.

Experiment C. Binding gold nanoparticles to carbon nanotubes through 16-nucleotide DNA molecules.

Samples 1 and 2 are aligned MWNT on gold foil, to which DNA (NT3') is chemically attached by the azidothymidine method. Sample 4 is unmodified, aligned MWNT on gold foil. Sample 3 is dispersed, oxidized nanotubes. Gold nanoparticles, or DNA-modified (Gold2A-SH3') gold nanoparticles, were added to the nanotube samples as indicated in the table below.

	Au-(Gold2A-SH3')	Au
Nanotubes-NT3'	sample 2	sample 1
Nanotubes	sample 4	sample 3

Method: The carbon nanotube samples were equilibrated in ~50µl ExpressHyb (hybridization solution from Clontech (Palo Alto, CA)) at 50°C for 30 minutes. The ExpressHyb solution was exchanged twice. Sample 3 appeared to swell in volume, with the nanotubes becoming very dispersed, and forming a brownish-black solution. The other samples had nanotubes attached to gold foil, and did not become dispersed.

50µl of 5nM gold nanoparticle solution was added to each of samples 1 and 3, and 25µl of 17nM DNA-modified gold nanoparticle solution was added to each of samples 2 and 4. The mixtures were vortexed, centrifuged, and left to slowly rock at 37°C for 20 hours.

Pale red supernatants containing free gold nanoparticles or free DNA-modified gold nanoparticles were removed from samples 1, 2 and 4. The samples were washed with 5µl of 2X SSC, 0.1% SDS, centrifuged, and supernatants were again removed. This washing procedure was repeated twice more, at which stage supernatants were colourless. Finally, the samples were washed twice with 0.1M NaCl,

10mM sodium phosphate buffer. Supernatants remained clear.

Sample 3 was centrifuged for 5 minutes. Most nanotubes settled to the bottom of the tube. The black-brown supernatant was removed and the remaining nanotubes were washed as described above, but with extended times for centrifuging.

50µl 0.1M NaCl, 10mM sodium phosphate buffer were added to all four samples, in preparation for imaging by TEM.

TEM images of each sample are shown in Figure 3. Gold nanoparticles pepper the surfaces of nanotubes in Sample 2 (Figure 3(a)), revealing that the azido-thymidine method results in chemical attachment of DNA all over the surfaces of nanotubes, as expected. There are no gold nanoparticles on nanotubes in the control sample 1 (Figure 3(b)), indicating that gold nanoparticles are not attracted to DNA-modified nanotubes under the experimental conditions used. Occasional nanotubes in control Sample 4 have a very small number of gold nanoparticles on their surface, indicating that DNA on the gold particle may be physically adsorbed on the nanotubes; one such nanotube is shown in Figure 3(c). These gold nanoparticles do not seem to be as discrete as those in Figure 3(a), but more blurred as if they have partially "melted". There are gold nanoparticles scattered through Sample 3 (Figure 3(d)) which consists of the oxidized nanotubes which formed a brownish-black mixture in ExpressHyb. These gold nanoparticles do not appear to be peppering the surfaces of nanotubes, as in Sample 2, but rather are mainly caught up in what appears to be amorphous material.

Experiment D. Binding gold nanoparticles to carbon nanotubes using DNA molecules longer than 16 nucleotides.

The above experiment was repeated, this time including longer DNA molecules on both the nanotubes and the gold nanoparticles. A T_{19} (= TTTTTTTTTTTTTTTTTTT)

extension on the DNA allows the remaining 16 nucleotides, which form the double helix and join the nanotubes to the gold nanoparticles, to extend away from the surface of the nanotubes and gold nanoparticles, and hence further into solution. The experimental procedure was similar to that for Experiment C, except that the washings with 2X SSC, 0.1% SDS were replaced by three washings with 0.1M NaCl, 10mM sodium phosphate buffer. The final washings were colourless.

Samples I, J and K are multi-walled aligned carbon nanotubes on gold foil, to which DNA (NT3'-T_{19}) is chemically attached by the azidothymidine method. Samples L, M and N are unmodified, aligned multi-walled nanotubes. Gold nanoparticles, or DNA-modified (either Gold2A-SH3' or Gold3A-SH3') gold nanoparticles, were added to the nanotube samples as indicated below. Note that the DNA molecules Gold2A-SH3' and Gold3A-SH3' have 16 and 35 (=16+19) nucleotides, respectively.

	Au-Gold2A	Au-Gold3A	Au
Nanotubes-T_{19}-NT3'	Sample I	Sample J	Sample K
Nanotubes	Sample L	Sample M	Sample N

TEM images are given in Figure 4. These show gold nanoparticles all over the surfaces of nanotubes in Samples I and J, indicating that DNA on the gold nanoparticles is able to bind complementary strands which are chemically attached to the nanotubes. In the controls, there are no gold nanoparticles, indicating that gold nanoparticles are not attracted to DNA-modified nanotubes (Sample K), DNA-modified gold nanoparticles do not bind non-specifically (through physical adsorption) to unmodified nanotubes (Samples L and M), and gold nanoparticles do not attach to unmodified nanotubes (Sample N), under the experimental conditions used. The

azido-thymidine method of chemical attachment of DNA to nanotubes results in DNA being placed all over the surfaces of the nanotubes, as indicated by the gold nanoparticles in Samples I and J. These results essentially confirm all of those obtained in Experiment C.

Experiment E. Binding DNA-modified gold nanoparticles to DNA-modified Single-Walled Carbon Nanotubes.

DNA (NT3'-$NH_2$3', NT3' with an amine linker on the 3' end) was reacted with single-walled nanotubes (commercial sample from ILJIN) which had been functionalised with carboxyl groups on the tips. Using this reaction, the DNA was expected to become chemically attached to the tips of the nanotubes via an amide bond.

Approximately equal volumes of DNA-modified SWNT (Samples C and D) and un-modified SWNT (Samples E and F) were pipetted into eppendorf tubes, and excess water was removed. 50µl ExpressHyb was added to each sample. (It appeared that the ExpressHyb partially solubilised material in each sample (this could be amorphous carbon)). The samples were gently rocked for about 15 hours at room temperature, and then at 45°C for 1 hour. The samples were centrifuged at 10,000rpm for 4 minutes. The ExpressHyb was removed, and 50µl of an ~10nM freshly-made mixture of DNA-modified gold nanoparticles in ExpressHyb was added to samples C and E, while a freshly-made solution of unmodified gold nanoparticles in ExpressHyb was added to samples D and F, as indicated below.

	Au-(Gold2A-SH3')	Au
SWNT-NT3'	Sample C	sample D
SWNT	Sample E	Sample F

The samples were left to sit at 45°C for 15 min, and then were gently rocked at room temperature for 8 hours. The samples were centrifuged at 10,000rpm for 3 minutes, and supernatants were removed. The supernatant

removed from Sample C was colourless (indicating that most of the DNA-modified gold nanoparticles were bound to the nanotubes), while the supernatants from Samples D, E and F were pink (indicating that most of the gold nanoparticles had not reacted with the nanotubes).

The samples were washed three times with 80µl of 0.1M NaCl, 10mM phosphate buffer to remove excess ExpressHyb and unbound gold nanoparticles.

TEM images of the samples are shown in Figure 5. The TEM images reveal that the single-walled nanotubes are not isolated, but rather form bundles of nanotubes which in turn are matted with other bundles. A large number of discrete gold nanoparticles are seen in sample C (Figure 5(a)), while a few isolated gold nanoparticles are seen in samples E (Figure 5(c)) and F (Figure 5(d)). The much larger number of gold nanoparticles in Sample C, compared with the controls, indicates that DNA chemically attached to the nanotubes is binding specifically to the DNA on the gold nanoparticles. The few nanoparticles in samples E and F may be physically trapped in the nanotube mats. Since the nanotubes are bundled, it is not possible to locate precisely the tip of any single nanotube, and hence to see if the gold nanoparticles are positioned there. However, the TEM image of sample C (Figure 5(a)) is quite different from those of samples I and J in Figures 4(a) and 4(c), respectively, where the DNA is attached to the surfaces of multi-walled nanotubes, and so it is likely that DNA is on the tips of the single-walled nanotubes in sample C.

Experiment F. Visual assay to determine if DNA physically attached to nanotubes by *in situ* synthesis from an oligothymidine linker is able to hybridise its complementary strand.

In order to check if any physically adsorbed DNA remained attached to the nanotubes (after *in situ* synthesis, removal of protecting groups from the bases and

extensive washing), and to see if it was in a state where it could bind its complementary strand, the unmodified nanotubes that had been through *in situ* DNA synthesis (Sample 1), the azidothymidine-modified nanotubes that had been through *in situ* DNA synthesis (Sample 2), and unmodified, aligned multi-walled nanotubes that had not been through the DNA synthesis (Sample 3) were assayed using the gold-nanoparticle/TEM assay as described below. Briefly, each sample was pre-incubated in three exchanges of ExpressHyb at 37 °C, before a freshly-prepared solution of ExpressHyb containing gold-nanoparticles modified with DNA (Gold2A-SH3') was added to each. For further controls, a freshly prepared solution of ExpressHyb containing gold nanoparticles (without DNA) was added to separate samples of each of the above which had also been pre-incubated in three exchanges of ExpressHyb at 37 °C. All six samples were left to rock gently overnight at 37 °C. Then, the ExpressHyb and unreacted gold-DNA or gold nanoparticles were removed, and the samples were washed three times with 100µl of 0.1M NaCl, 10mM sodium phosphate buffer, before resuspending in 50µl 0.05M NaCl, 10mM sodium phosphate buffer, and imaging by TEM. The TEM images are shown in Figure 6. The similar appearance of gold nanoparticles in Figure 6(a) (sample 1, DNA physically attached to nanotubes) and 6(c) (sample 2, DNA chemically attached to nanotubes) reveals that DNA synthesized on unmodified, aligned multi-walled nanotubes is sufficiently strongly attached to the nanotubes by physical adsorption to remain in place through stringent and vigorous washings, and heating for prolonged periods at 37 °C, and that this DNA is able to bind to its complementary strand on the gold nanoparticles. No gold nanoparticles appear in other controls.

It is possible that some of the thymidine nucleotides, which would form part of the T19 chain in the full-length molecule NT3'T19, may be laying against the walls of the nanotubes in Sample 1, and hence would not be

available for hybridization to complementary DNA. This was not tested here, since only 16 nucleotides at the 5′ end of the DNA on the nanotubes was used for base pairing with DNA on the gold nanoparticles.

EXAMPLE 4: THE DEVELOPMENT OF SELF-ASSEMBLED, NANO-SCALE DEVICES.

This experiment demonstrates the use DNA to precisely locate carbon nanotubes between two gold electrodes, and to measure the current-voltage properties of this completed, self-assembled circuit.

Aligned, multi-walled carbon nanotubes are prepared by pyrolysis of iron(II) phthalocyanine (FePc) under Ar/H_2 at 800-1100°C, checked for amorphous carbon impurity using Scanning Electron Microscopy, cleaned-up by plasma treatment, and transferred from the quartz onto a gold-foil substrate (as described by Li, D.-C., Dai, L., Huang, S., Mau, A.W.H. and Wang Z.L. (2000) Chem. Phys. Lett, 316, 349-355). Single-walled nanotubes, purchased from two commercial sources (Iljin Nanotech Co. Ltd. (Korea), and Carbon Nanotechnologies Inc. (Houston, Texas)) are checked for purity using TEM or SEM, cleaned up, tested in various solvents and given ultrasonic treatments for various times to de-bundle and cut the nanotubes into lengths less than 1 μm. The quality of the nanotube samples so prepared are checked using SEM and/or TEM, as appropriate. The tips of the single-walled nanotubes are functionalised with carboxylic acid groups by refluxing the nanotubes in HNO_3/H_2SO_4 solution.

DNA oligonucleotides with an amino linker (NT3′-$NH_2$3′, and NT3′-$NH_2$5′), and with a thiol linker (Gold2A-SH3′) are synthesized using the Applied Biosystems DNA synthesiser. The oligonucleotides are labelled with P^{32} to check purity. The oligonucleotides with amino linkers are chemically attached to the sides of unmodified SWNT using the azido-succinimide method. These oligonucleotides with amino linkers are also chemically attached via an amide

bond to the tips of SWNT functionalised with carboxyl groups. Oligonucleotides (NT3'-T19) are built up from azido-thymidine (previously bound by photochemical reaction to the walls of the MWNT) using the Applied Biosystems DNA synthesizer. Oligonucleotides (NT3'-T19), physically attached to the MWNT, are also built up by synthesis in the Applied Biosystems DNA synthesizer, starting with unmodified MWNT.

The MWNT and SWNT samples are analysed by XPS to determine the extent of chemical and/or physical attachment of DNA. Gold nanoparticles, of diameter approximately 15nm are prepared as described above. The oligonucleotide with a thiol linker, Gold2A-SH3', is bound to the gold nanoparticles as described above. The locations of the DNA molecules on the MWNT and SWNT are determined by hybridizing these DNA molecules with the complementary DNA molecules on the gold nanoparticles and visualizing the locations of the gold nanoparticles using TEM, as described above.

A gold coating is sputtered over a silicon substrate. The gold is patterned to create a simple electrode system which serves as the test system plus control. Non-specific sticking of DNA-modified carbon nanotubes to the silicon substrate is checked by imaging with AFM. Where necessary, the silicon substrate is covered with a self-assembled monolayer of molecules with exposed negatively charged functional groups in order to reduce non-specific binding of DNA-modified nanotubes.

Using a mask, single-stranded DNA molecules (pre-made, with thiol linker) are spotted on to the gold electrodes in the desired locations. The mask is removed, and the chip is dipped into MAAD (mercapto-acetic-acid-dimer) to cover remaining gold surfaces with a negatively-charged self-assembled monolayer. The carbon nanotubes are positioned across the gold electrodes using hybridization between the DNA on the modified nanotubes and their complementary DNA molecules bound to the gold

electrodes. The control is unmodified nanotubes. AFM is used to image the results.

The positioning of nanotubes in a defined pattern on the gold electrodes demonstrates in principle that DNA may be used as an agent for self-assembling devices.

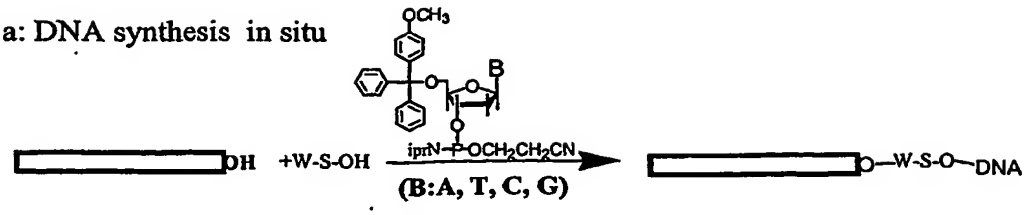
Current-voltage curves for the two-electrode system spanned by a nanotube or group of nanotubes are measured. The measurements are repeated for the control where two electrodes are not spanned by a nanotube.

If conductance is not sufficiently high, refinements may be made in subsequent work by improving the conductance of the DNA (for example by using metal-bound phosphorothioated DNA, or by incorporating gold nanoparticles at the junctions, or by silver-coating the DNA).

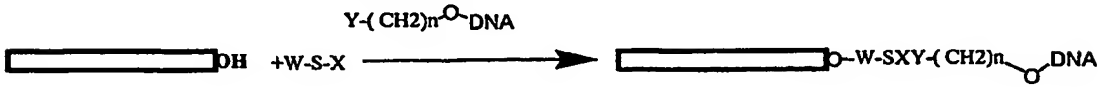
EXAMPLE 5: DNA SENSOR (ELECROCHEMICAL SENSOR).

The procedure to assemble an electrochemical sensor is described below. This procedure involves the use of an electroactive indicator, which intercalates the double stranded DNA. Transition metal complexes such as Co and Ni complexes, or reagents such as Daunomycin hydrochloride (Marrazza G. *et al.*, 1999, Biosensors & Bioelectronics 43-51) can be used as intercalators. The immobilized DNA on the electrode, which can be made from aligned MWNTs or mats of SWNTs, is incubated in the sample solution containing the target DNA and the electroactive indicator for 10 min and then washed with buffer solution using 0.02 M phosphate buffer pH 7.0, with 0.05 M NaCl. The potential value of the anodic peak for the transition metal complex or daunomycin obtained in differential pulse voltammetry is used to detect the presence and the amount of the complementary DNA. Alternatively, the hybridization process is monitored with a chronopotentiometric transduction mode.

1a: DNA synthesis in situ

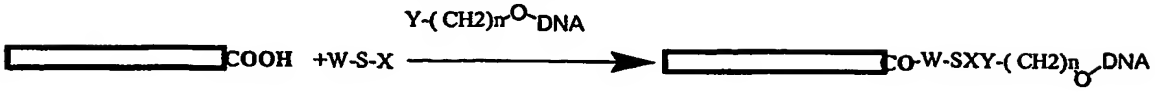


1b: Chemical reaction with presynthesised DNA

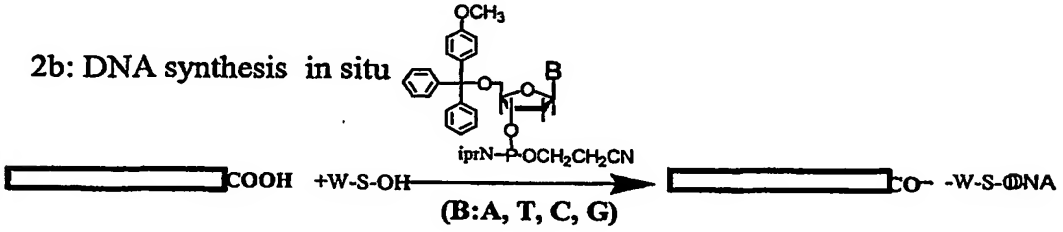


Scheme 1

2a: Chemical reaction with presynthesised DNA

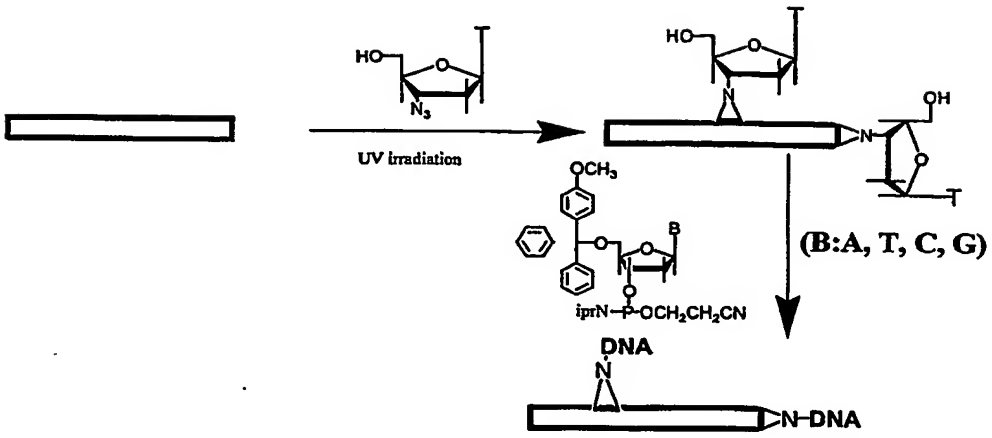


2b: DNA synthesis in situ

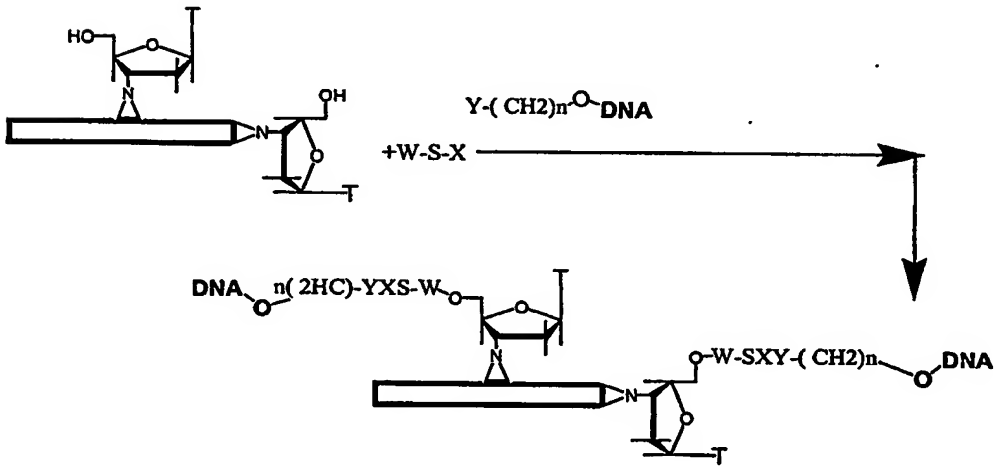


Scheme 2

3a: DNA synthesis *in situ*

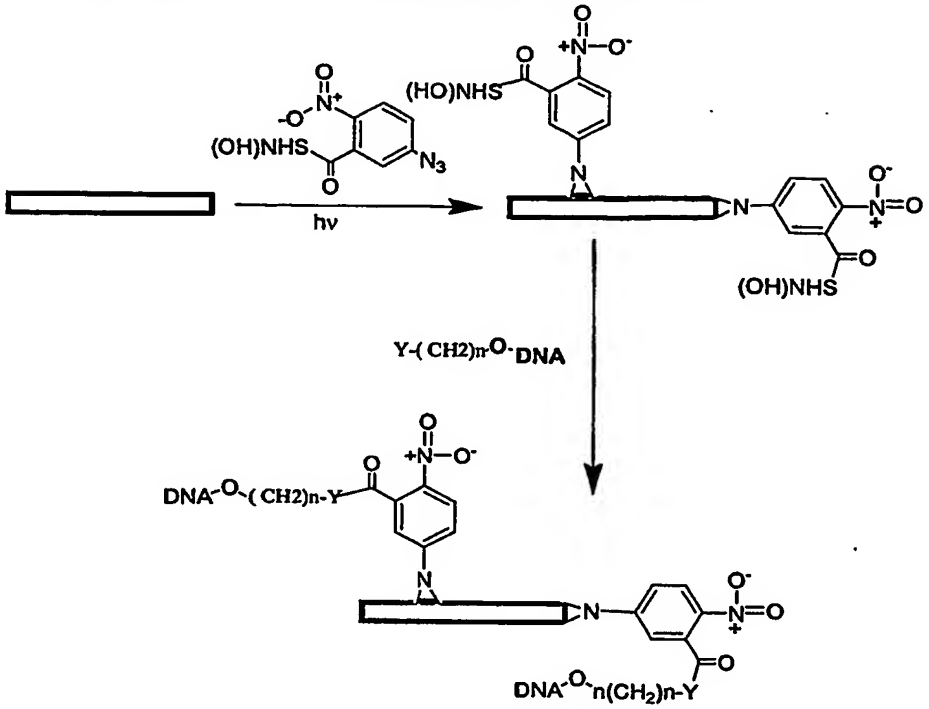


3b: Chemical reaction with presynthesised DNA

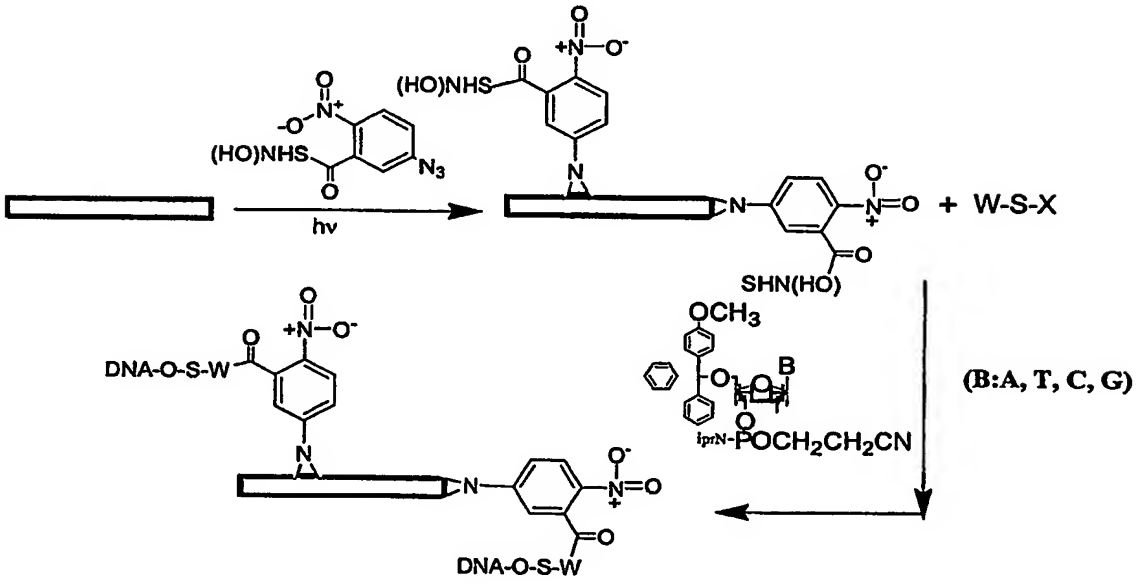


Scheme 3

4a: Chemical reaction with presynthesied DNA



4b: In situ synthesis of DNA



Scheme 4

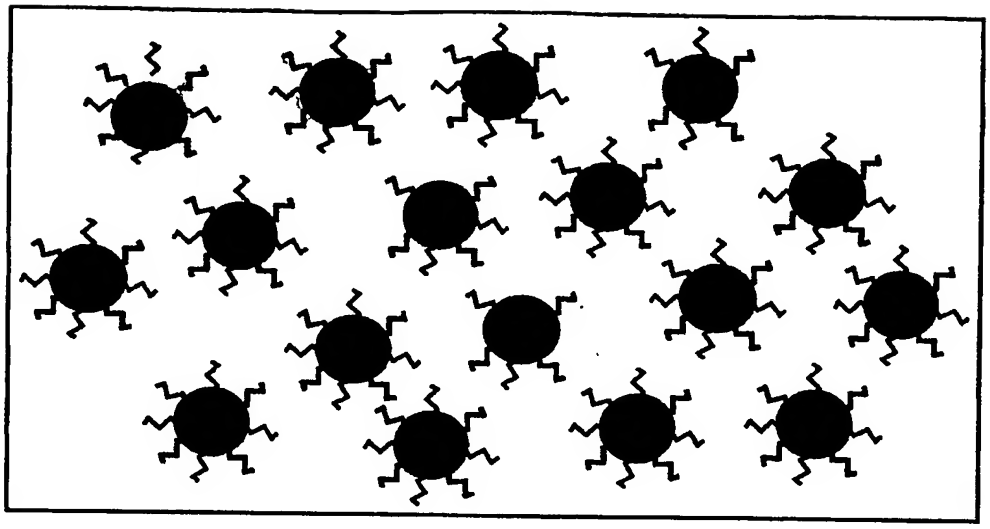


Figure 1

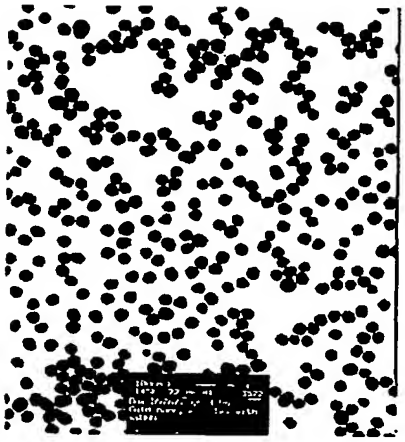


Figure 2

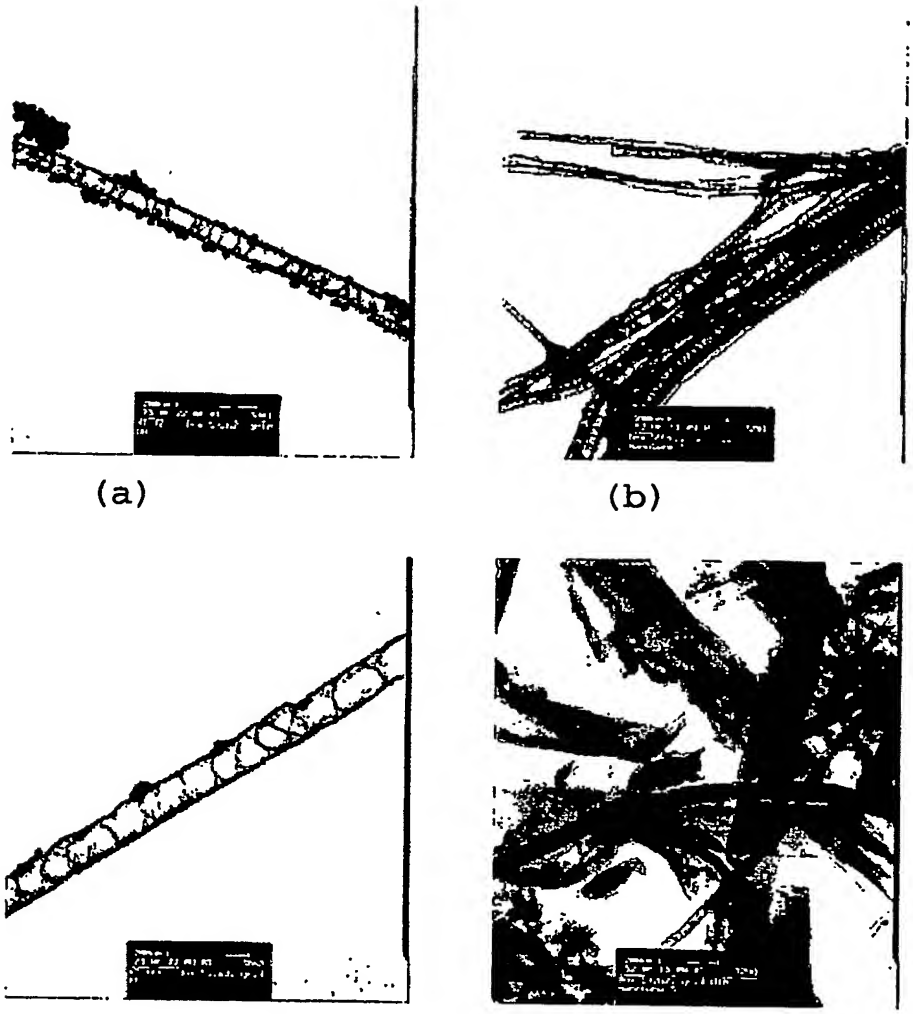


Figure 3

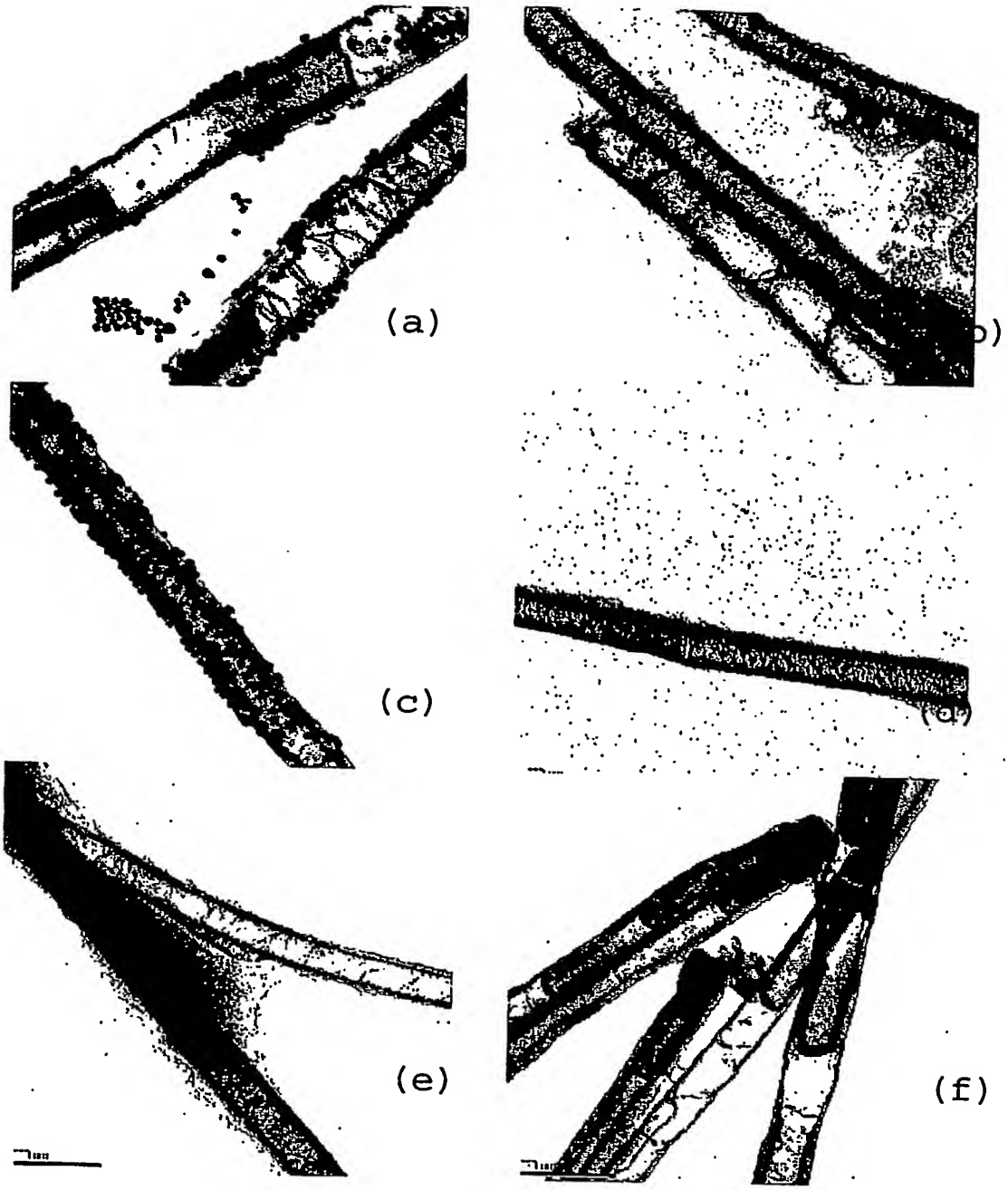


Figure 4

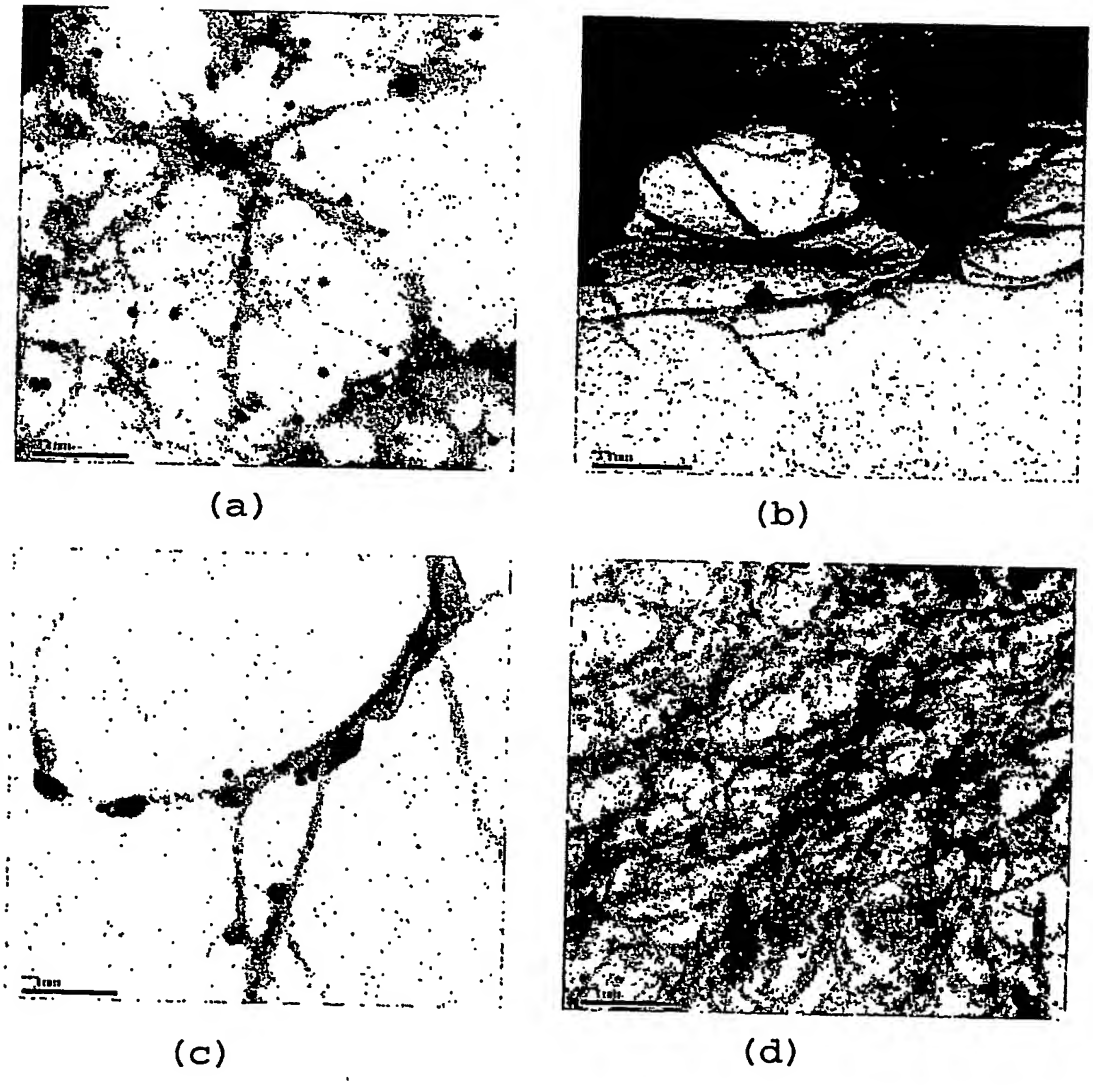


Figure 5

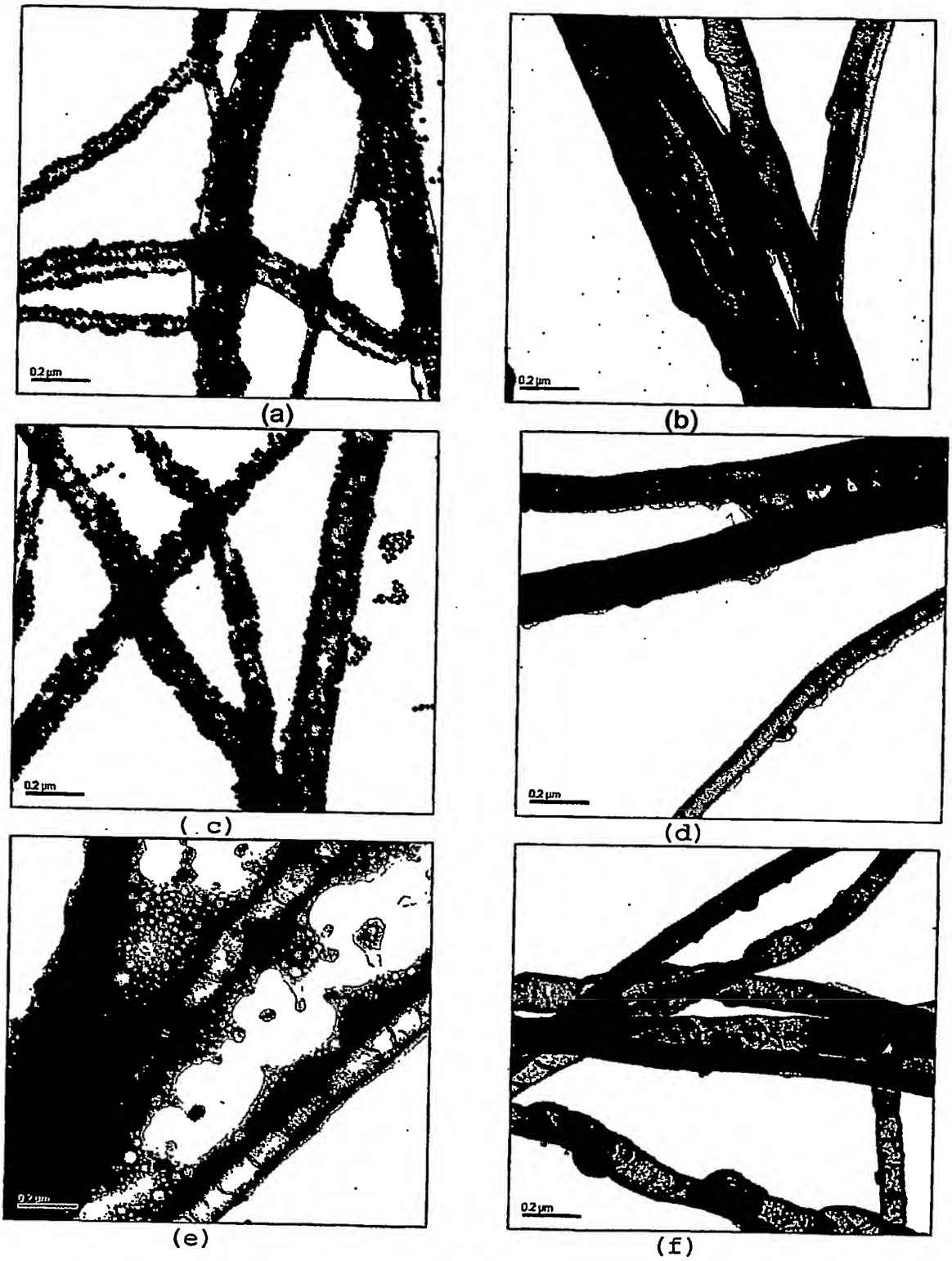


Figure 6